PHAGOCYTOSIS AND IMMUNITY

AN

EXPERIMENTAL RESEARCH

by

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Ever since the days of Hippocrates and Galen, efforts have been made to find out the cause of natural and acquired Immunity. It is, however, only within the last quarter of a century that much light has been thrown on this problem. New properties of the immune organism have been discovered and many of them have been accredited with the cause of the Immunity itself. Although up to the present day no entirely satisfactory solution has been arrived at, two theories have arisen out of these discoveries which appear to explain Immunity better than any other. These are the Humoral Theory and the theory of Phagocytosis.

The Humoral Theory was first introduced by Fodor, was worked at especially by Buchner (1), Bordet (2) and Pfeiffer (4) and then reached a great importance through the system built up by Ehrlich. Those who hold the humoral theory believe that immunity consists in the presence/

presence of various substances in the body fluids which deal with the bacteria and their products outside of and apart from the cell. Some of these, such as Bacteriolysins and Agglutinins may exist either in the Normal or in the Immune Organism. Others again which have been produced solely in response to a particular stimulus are believed only to exist in the Immune body fluids. Such are Antitoxin, precipitin and the theoretical Anti-aggressin of Bail (26), for example. Of all these substances the most discussed has been the Bacteriolysin. It consists of two parts, the one of which, the Immune body, Amboceptor or Sensibilatrice, fixes itself on to the bacteria without producing evident change. The other, according to Ehrlich and his school, unites itself by a chemical combination with the Amboceptor, or according to Bordet unites itself immediately to the cell which has been prepared for it by the mordantlike Sensibilatrice. This second part is the active, or lytic part, and is called by Ehrlich the Complement, by Bordet the Alexine or Cytase. There has been much discussion as to whether or not this Bacteriolysin or Antibody is the responsible cause of Immunity, also whether these two substances of which it is composed can display other activities beside that of dissolving bacteria.

The second Theory of the cause of Immunity is that of phagocytosis. Metchnikoff (6) discovered that the

leucocytes /

leucocytes were able to ingest bacteria, that they were increased in immune blood, and that the serum contains substances which influence Phagocytosis. He believed that these substances stimulated the phagocytes. In 1895 Denys and Leclef (7) drew attention to these substances, which they suggested acted on the bacteria, and were different to Amboceptors. In 1902 Leishman (8), who had been influenced by this work, devised a method by which he hoped to turn a scientific fact to practical account by comparing the phagocytic power of normal and morbid serum. In 1903 Wright, taking advantage of Leishman's quantitative method, which he improved, demonstrated that these substances in the serum concerned with phagocytosis act not on the leucocytes, but on the bacteria. This fact is now generally held, although the stress which Wright (9), and his followers Bulloch and Atkin (14) laid upon the fastness of the combination between the Opsonins, as he called them, and the bacteria, does not appear to be quite justified.

Although these two theories are in one sense rival theories, workers in the field of Immunity have not always maintained a strict distinction between them. It is true that there are many who hold the one theory in preference to the other, but this is not perhaps the most important line of cleavage. There are two great classes of biologists who look not only at Immunity, but also/

also at the construction of the cell itself, at nutrition, at life and death from entirely different standpoints. First Ehrlich (5) and his school believe that the cell is one great chemical molecule with side-chains which are naturally present for purposes of nutrition, but may act for the protection of the body against bacteria which are also molecules with side-chains. The processes of Immunity are purely chemical to be explained by "unsaturated affinities", and "neutralisation" like acid and base. Jacoby and others have actually attempted to precipitate toxin-antitoxin compounds and to analyse them, so great was their faith in the firmness and stability of these combinations. This theory is not accepted by many chemists, who prefer, like Arrhenius (15) etc. to explain as much as they can by purely or partly physical laws. However, the influence of the school of Ehrlich so pervades the literature of Immunity, that it is necessary to use his nomenclature and to argue on somewhat hypothetical grounds which is rather apt to hinder a straight-forward and practical issue.

When Wright (9) made this discovery that the substances concerned in phagocytosis acted on the bacteria, the question naturally arose of whether they had any connection with Agglutinin and Bacteriolysin. To this day, although much work has been done, the question has not been perfectly settled.

Dean/

Dean (16) has pointed out that Agglutination raises phagocytosis, specific or non-specefic. Hektoen and Ruediger (19) believe in an Agglutinin-like structure of Opsonin which may in their opinion form an agglutinoid-like or "opsinoid" form by heat. Bail (25) mentions an instance in which he noticed Opsonin and Agglutinin disappear together in the body-fluids. Keith (52) remarks on the rapidity of phagocytosis in contrast to the slow movements of leucocytes and suggests that an agglutination of the bacteria or red cells may take place in the direction of the leucocytes to explain this paradox. Analogy, however, between Agglutinin and Opsonin ceases entirely here. Opsonin to Tubercle is very much reduced above 51° and in many cases is destroyed at 56° or almost entirely destroyed, while Agglutinin is destroyed above 62° about 65°. Amako (30) has published charts of daily records taken in many cases of cholera, comparing the bacteriolytic, phagocytic and agglutinising power of the serum. The three curves were quite independent, one power being perhaps absent when the others were present. Barratt (53) has found haemo-opsonin absent in a serum which could agglutinate.

Whether Opsonin is or is not identical with Bacteriolysin or one of its parts is a matter of fundamental importance, and for this reason. Bacteriolysis and Phagocytosis both appear very practical methods of protection./

protection. Their application is, however, limited. If it were to be proved that both were caused by the same immune substance which could display such very varying activities according to circumstance, the stress which has been laid upon the importance of the Amboceptor and Complement would appear to be better justified, than the work of Bail and Peterson (24) would perhaps allow. A fresh impetus would be given to the study of these limitations, perhaps as a way to their practical remedy.

It has been shown that Bacteriolysis is often wholly absent with an immune serum. Bordet and Gengou (3), however, have shown that the Amboceptor and Complement may be present in the serum, but may not act, either on account of the fact that the Bacterium is not capable of being bacteriolysed, e.g., Tubercle (Wright) (11) or Streptococcus (Neufeld and Rimpau) (31) or because the Bacteriolysis is prohibited on account of some deviation of the Complement or other extraneous circumstance. Sawtschencko (37) has suggested that the Immune body acts in minuter quantities as a phagocytic agent, and in larger amounts bacteriolytically. Wright (10) does not hold that the two bodies are identical, because Opsonin may be present in serum when Bacteriolysin is absent, and when co-existent, their measure is not parallel. He has separated Bacteria into four classes, three of which show/

show different behaviour to Bacteriolysis, and Phagocytosis, and the fourth is equally insensitive to both. Bacteria of this last diphtheria group have, however, been shown, Hamilton and Horton (72) etc. to be sensitive to Immune Opsonin. In the other three classes sensitiveness to Phagocytosis is never stated to be less, thus perhaps supporting Sawtschencko(37) . Against this, the charts of Amako (30) already mentioned, give a bacteriolytic curve generally higher than the phagocytic and with the phagocytic occasionally at nil when the bacteriolytic was high. Fornet and I (38) have recorded that for one particular strain of Paratyphoid normal human serum has comparatively slight opsonic power, but intensely strong bacteriolytic. It follows, therefore, that the argument is unjustified that Opsonin may be present in non-bacteriolytic serums because smaller quantities of the Immune body will suffice for phagocytosis, nor again is the reverse possible.

Wright has claimed for Opsonin that it is the cause of Immunity, just as Neufeld (31) for the Bacteriotropins, and Bail (26) for the Anti-aggressins. It is especially in the case of Tuberculosis that Wright has made this claim, a disease in which Immunity appears so very doubtful. Tuberculosis seems to stand quite alone among other infectious diseases. The bacillus cannot be bacteriolysed, Agglutination and Precipitation tests, from/

from which at one time much was expected, have been largely given up. Instead of producing an Anti-aggressin, the toxins appear instead to cause an over-sensitiveness. The only toxic substance which analysis of the bacillus can discover is the Tuberculin-acid too simple a chemical to produce Anti-bodies. In spite of this Wright asserts that we have still Opsonins to fall back upon, and in Opsonins he places very great hope.

Bacteriolysis has certainly a much more limited field than phagocytosis. There are, however, undoubtedly reasons for regarding the theory that Opsonin is the true immune substance with some doubt. Bulloch and Atkin (14), Urwick (40), Lawson and Stewart (41) Meakin and Wheeler (42) and others have reported acute cases of tuberculosis have commonly or not uncommonly high indices.(43) Fornet has found, as also I myself independently, high indices a few days before death. Fornet and I have been struck by the fact that in experiments carried out by us two years ago guinea-pig serum was as a general rule several times human in opsonic strength when guinea-pig leucocytes were used. I have since noticed with human leucocytes they are about equal. Allowing for the contention of Bächer (44), and Hamburger and Hekma (46) which I endorse, that phagocytosis is limited where heterogeneous cells and serum are used, this still places guinea-pig serum considerably higher than human in opsonic/

opsonic strength. Yet guinea-pigs are so fatally susceptible to tuberculosis. Bail (27, 28, 29,) Béraneck (47) and many others have declared that it is absolutely impossible to immunise a guinea-pig against Tuberculosis. Levy and others have believed themselves successful, but I do not know of any results carried on longer than two years. It is generally known that an occasional live bacillus exists in Koch's T.R.Tuberculin. Béraneck has injected guinea-pig with T.R. and some have died of tuberculosis, so susceptible is this animal. Yet the guinea-pig serum has a remarkably high opsonic power. Then again we have often a raised index during pregnancy followed by the rapid increase of the disease which is apt to follow a recent pregnancy in a tuberculous subject. This is then how we stand at present as regards phagocytosis and Immunity. Bacteriolysin and Opsonin explain Immunity better than any other immune substances. Opsonin may, however, exist without Immunity and Immunity without Bacteriolysin. I have attempted in the following experiments to discover--

I. Whether Opsonin is identical with Bacteriolysin and

II. the relation between Opsonin and Immunity.

I A considerable amount of work has been done on this question, and out of it very conflicting results have appeared.

Wright/

Wright and Douglas (9) believe for reasons given above, that Opsonin is a new substance of simple form which has no connection with Bacteriolysin.

Bulloch and Atkin (14) support this belief for the same reasons, also because they believe Opsonin can be absorbed at 0,° and is destroyed at 56.°

Hektoen and Ruediger (19) believe that Opsonin has a simple structure which is converted by heat into an opsinoid, and therefore cannot be Bacteriolysin.

Löhlein (50) holds that Opsonin is a new substance, because he has failed to reactivate heated serum by fresh, he finds Opsonin destroyed at 56° and absorbed at 0.°

Hamilton and Horton (72) believe too that Opsonin is new, and also simple.

Fornet and myself (38, 39) hold this view too, because we have failed to reactivate heated serum and have managed to separate Opsonin and Complement.

Keith (52), and Barratt (53) regard Haemo-opsonin as a new, and simple substance.

A group of authors believe that normal Opsonin is identical with Complement, these are Levaditi and Inmann(76) Levaditi and Koessler (77), Muir and Martin (82), Haet-jens (54), Sleeswijk (66) and Hata (109). They believe this because under certain conditions, such as absorption with Yeast cells, complement deviation of all kinds which they have attempted has led to the disappearance of/

of Opsonin as well as Complement. Could one exception to this be discovered their arguments must of necessity fall to the ground.

Sawtschencko (37), and both Levaditi and Dean (16) at one time have ascribed to the amboceptor the whole of the opsonic effect in immune serum, Dean apparently also for normal serum.

In a later paper Dean (18) changes his position, he now believes that while Amboceptor is effective alone, its power is much enhanced through the presence of Complement. He does this because he has been successful in obtaining a few instances of apparent Reactivation.

Various other authors think that Opsonin and Bacteriolysin are identical, or at least that Opsonin consists of two parts, Amboceptor, and Complement.

Neufeld and Hüne (35) believe this of Normal Serum but not of Immune.

Levaditi (77) believes this of Immune, but not of Normal.

Cowie and Chaplin (55) of Normal Serum, but unfortunately they chose Staphylococci as bacteria, and it is difficult to be certain that any Serum is really normal to this organism so common are staphylococcic infections.

Caulwell (56) holds this theory for both Normal and Immune, but publishes results proving the converse.

Meyer (57) thinks that Normal Serum is composed of

an/

an Amboceptor and Complement, but he used Paratyphoid which is bacteriolysed, and incubated for an hour, he used sedimentation tubes, instead of Wright's method, and he only roughly judged of phagocytosis by the eye.

Böhme (59) is able to reactivate Immune Serum, in one instance normal.

Browning (58) holds this theory of Normal Serum, but is forced to regard the Complements as not identical.

Sleeswijk (66) and Hata (109) believe in this Formation for immune Serum.

In order to discover whether Normal Opsonin is identical with Bacteriolysin or either of its parts, it is well to consider each possibility separately.

A. Whether Normal Opsonin and Complement are identical.

B. Whether Normal Opsonin and Amboceptor are identical.

C. Whether Normal Opsonin consists both of Complement and Amboceptor.

A. Normal Opsonin and Complement.

To discover whether Opsonin is identical with Complement it is necessary to compare their characteristics as regards the influence of

(1)/

(1) Heat.

(2) Cold.

(3) Dilution.

(4) Chemical Reaction.

(5) Time.

(6) Origin.

(7) Absorption.

(8) Dialysis.

(1) The influence of Heat. a) with Tubercle Bacteria.

Exp.	Normal Guinea-pig Serum	N.G.S. Heated 56°	N.G.S. Heated 60°	0·85% NaCL Alone
Table 1	2·7		0·4	
3	3·76		0·54	
4	1·76		0·5	0·55
5	3·4	1·8	0·96	0.84
	4·2	3·44	1·0	0·84
6	5·5		0·94	0·84
8	2·8	1·34	0·78	0·62
9	2·7	1·18	0·9	
38	1·12		0·1	0·0
65	1·6	0·52	0·28	0·1
66	1·62		0·2	0·03

Experiment

64/

Experiment	Normal Rabbit Serum	N.R.S. Heated 56°	N.R.S. Heated 60°	0.85% NaCL Alone
64	3·0	0·4		0·06
63	4·0	0·9	0·2	0·1
62	1·3	0·16	0·06	0·06

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	0·85% NaCL Alone
10	2·03	0·2	0·55	0·33
11	3·85	0·23	0·33	0·22
12	3.45	0·62	0·12	0.13
13	2·85	0·23	0·2	0·2
14	2·66	0·3	0·23	0·03
15	4·76	0·7	0·1	0·22

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	0.85% NaCL Alone
16	4·66	0·53	0·2	0·1
17	4·13	0·5		0·2
20	3·56	0·23	0·33	0·2
21	4·36	0·5		0·2
"	5·0	0·4		0·2
22	1·23	0·23		--
23	2·65	0·13		0·1
24	3·5	0·35		0·1
38	1·66	0·0		0·0
"	1·0	0·23		0.0
44	10·0	0·8		0·46

46/

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	0·85% NaCL Alone
Table 46	4·0	1·0	1·0	0·6
47	2·5	0·2		0·1
48	3·0	0·3		0·26
49	2·94	0·16		0·1
50	2·85	0·2		0·2
51	2·7	0·2		0·08
54	2·5	0·2		0·1
57	1·56	0·4		0·03
58	2·5	0·06		0·06
60	2·13	0·3		0·1
62	1·5	0·25	0·1	0·06
63	5·4	0·2	0·1	0·1
64	4·09	0·2		0·06
65	1·54	0·52	0·26	0·1
66	1·2	0·06		0·03
67	1·27	0·06		0·03
"	0·9	0·1		0·03
69	0·82	0·2		0·06
"	0·73	0·06		0·06
70	0·7	0·03		0·0
"	0·73	0·03		0·0
71	1·55	0·1		0·03
72	1·0	0·1	0·03	0·0
73	1·23	0·0	0·03	0·0
74/				

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	0·85% NaCL Alone
Table 74	1·5	0·25		0·0
75	1·3	0·08		0·0
76	5·4	0·2	0·1	0·1

b) Pyocyaneus Bacteria

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	1·2% NaCL Alone
80	14·5	6·46	3·86	0·8
81	3·16	0·4		0·03
"	---	0·2		0·03
"	---	0·93	0·2	0·03
"	---	0·9	0·2	0·03
"	---		·2	0·03
82	3·6	0·6		0·1
83	0·83	0·2		0·03
84	1·6	---	0·6	0·02
"	---	0·43	0·4	0·02
"	---	---	0·6	0·02
"	---	---	0·43	0·02
85	1·8	0·4		0·1
86	11·3	0·8		0·4
87	1·1	0·6		0·3
88	12·13	2·15	---	0·3
"	---	1·73	1·43	0·3
"	---	1·73	1·3	0·3

88 continued/

Experiment	Normal Human Serum	N.H.S. Heated 56°	N.H.S. Heated 60°	1·2% NaCL Alone
Table 88	---	2·8	2·4	0·3
"	---	---	3·6	0·3
89	5·2	1·7	0·8	0·5
"	---	2·1	1·2	0·5
"	---	1·63	1·43	0·5
"	---	1·2	1·45	0·5
90	10·0	2·85	2·8	1.8
"		2·3	2·08	1.8
"		2·33	2·5	1·8
"		2·0	---	1·8
"		2·8	2·05	1·8
"		3·4	2·0	1·8
92	3·8	0·3	---	0·0

Experiment	Normal Human Serum	N.H.S. Heated 51°	N.H.S. Heated 53°	0·85% NaCL Alone	
46	4·0	2·3	---	0·6	
57	1·56	0·25	---	0·03	
58	2·5	---	0·3	0·06	Tubercle
60	2·13	---	0·33	0·1	
62	1·5	---	0·2	0·06	

From these tables it will be seen that Opsonin to tubercle loses at 51°for half an hour half its strength, at /

at 53°about 87%. Pyocyaneus at 56°loses about 79%. Madsen and Fanulener (60) have shown that Complement at 51°for half an hour loses 66%, and at 53°for 12, 89%, and Bordet that 55°for half an hour it is totally destroyed. Dean (16), Hektoen and Ruediger (19) and Klien (62)etc., have also noticed this residue. Wright and Reid (12) think it due to irregular heating and spontaneous phagocytosis, and suggest the use of 1·2-1·5% NaCL which inhibits this. In view of the effect of varying salt solution on other immune processes I consider this condition hardly fair. Noguchi (61) finds that if serum be dried and powdered Opsonin can endure a temperature of 150°, and Complement of 135°.

(2) The Effect of Cold upon Opsonin

I have found that if serum be kept at 0°-3°for half an hour it requires warming afterwards at 37°, in order to recover its full activity. In the following experiments a portion of fresh serum never more than 0·3cc was cooled at 0°for half an hour and then left at room temperature for some hours before use.

Experiment/

Experiment	Normal Human Serum	N.H.S. cooled at 0°	
28	1·4	0·35	
40	2·98	2·33	
"	2·0	1·7	
"	3·0	2·53	
"	2·5	2·5	
"	2·0	2·1	
"	3·0	2·63	
49	2·94	2·36	
"	2·94	2·9	warmed at 37°
"	3·4	2·0	
"	3·43	2·2	
"	2·7	1·95	
50	2·85	2·0	
"	3.07	2.47	
"	3·3	2·0	
"	3·26	2·36	
51	2·7	2·2	
"	3·0	1·56	
56	1·44	1·3	
"	1·13		
"	1·3	0·8	
"	1·0	1·0	warmed at 37°
"	1·4	1·44	
"	1·0	0·9	

The/

The effect cannot be due to cooling of the leucocytes as the serum must have been at 10°-15° like the Control serum at the time of the experiment when mixed with the leucocytes, also the amount of Opsonic power wanting differs in the different serum. There does not exist to my knowledge any record of a similar behaviour on the part of Complement, although doubtless such a record would be difficult to obtain on account of the longer incubation allowed to Complement. Millar and Taylor (64) describe a somewhat similar experience with a colloidal film which they had cooled to 0°, and which required to be left over the night at 25°, in order to recover. They believed that the chemical nature of the film had changed

(3) The Influence of Dilution

I. In comparing Opsonin with Complement it is especially important to discover to what extent each can be diluted. Sleeswijk (66) has found both Normal Opsonin and Complement active up to a dilution of $\frac{1}{640}$ at which point both were lost. In the case of haemolytic Opsonin and Complement Keith (52) has been able to carry Complement only to $\frac{1}{50}$ while Opsonin was active at $\frac{1}{90}$. Wright (9) has diluted Opsonin to $\frac{1}{192}$, and Hektoen and Ruediger (19) to $\frac{3}{100}$ without comparing the effect on Complement. From the fact which Noguchi (61) has pointed out/

out that Opsonin acts best in a neutral reaction, while Complement prefers an alkaline, one would expect that the more serum is diluted with neutral NaCL solution, Opsonin would stand a better chance. Dean (18) who believes that Opsonin and Bacteriolysin are identical, yet uses diluted serum, in order that the phagocytic count, which in $\frac{1}{100}$ heated serum + $\frac{1}{30}$ normal is marked, should not be obscured by bacteriolysis which by such dilution is disposed of.

(a) With Tubercle Bacteria.

Guinea-pig's serum was diluted to various strengths up to $\frac{1}{10,000}$, and each dilution added to

1ccm red corpuscles 5%

1ccm Amboceptor $\frac{1}{50}$

and filled up to 3ccm.

Haemolysis occurred up to $\frac{1}{100}$, at $\frac{1}{500}$ there was a slight reddish tint. In presence of 0·1ccm Complement the Amboceptor was equal to $\frac{1}{5000}$. The Opsonic strength of the Dilutions was as follows--

$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{800}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{5000}$	$\frac{1}{8000}$	$\frac{1}{10,000}$	0·85%NaCL
3·33	5·7	3·13	4·7	4·22	5·22	3·0	4·32	3·4	1·9

The Opsonic experiments were incubated for a quarter of an hour, the haemolytic for three, and then twenty-four hours in the ice-chest. In order that the Opsonin might also have the benefit of longer incubation the following experiments were done with diluted human normal Serum./

Experiment	52	53	54	78	79
Time of Incubation	3 hrs.	5 hrs.	3½ hrs.	3 hrs.	3 hrs.
Undiluted Human Serum		2·7	4·9		
1/50	5·6	2·4		15·9	4·9
1/100	5·0	1·5		14·3	4·55
1/500	6·53	1·2		13·3	5·6
1/1000	5·52	1·2		14·1	3·83
1/5000	7·16	1·4		11·13	3·63
1/10,000	5·5	1·1	0·2	12·38	3·4
1/20,000	3·95	1·5	0·4	12·33	2·9
1/50,000	4·8	0·5	0·1	8·5	3·0
1/100,000	6·9	0·96	0·1	8·9	1·8
0·85% NaCL.	3·03	0·3	0·03	6·0	1·43

With either 0·1 or 0·2 Amboceptor I was unable to carry a trace of haemolysis farther than 1:50 of fresh human serum.

III. (a) With Tubercle Bacteria.

It is also important to discover the curve of dilution whether this varies for different bacteria, whether the fall is rapid at first or more gradual. Dean (16) has found with Staphylococci that the loss of Opsonin on dilution is proportional to the square root of the concentration. Marshall (65) with the same organism, believes phagocytosis to be a linear function of the logarithm/

logarithm of the concentration. It would be interesting were the dilution curve proved the same whatever the bacterium. The fact that Dean experiences a rise instead of a fall with slight dilutions as $\frac{1}{2}$ is explained by the discovery of Noguchi (51) that a neutral reaction is the optimum for opsonic activity.

Exp.	Undiluted Human Serum	$\frac{1}{20}$ diluted	$\frac{1}{4}$ hour's incubation 0.85% NaCl.
58	2·5	0·54	0·06
60	2·13	0·6	0·1
67	1·27	0·2	0·03
68	0·75	0·12	
69	0·82	0·08	0·06
70	0·7	0·2	0·0
71	1·56	0·36	0·03
72	1·0	0·3	0·0
73	1·23	0·22	
74	1·5	0·4	

Exp.	Undiluted Guinea-pig Serum	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	0·85% NaCL
1	2.7	2·42	0·6	1·1	
2	2·2	1·65	1·35	1·0	
3	3·76	2·2	1·9	1·3	
4	——	0·96	0·8	0·36	0·55
5	3·4	2·64	1·96	1·5	0·84
6	5·5	3·24	2·6	1·6	
8	2·8			0·78	0·62

b)/

b) With Pyocyaneus Bacteria

Exp.	Normal Human Serum	1/4	1/20	1/100	1/500	1/1000	1/2000	1/5000	1/10,000	NaCL
82	8·6	2·1	1·7	0·83	1·26	0·76		0 63		0·3
83	9·32	1·3	0·6	---	1·0	0·7		0 3		0·3
91	12·4	9·0	8·9	6·8	9·2	5·3	8·6	6 8	5·5	1·45
80	14·5	3·3								
81	3·16	1·0								
82	3·6	0·6								
84	1·6	1·0								
85	1·8	0·35								
86	11·3	0·5								
87	1·1	0·5								
88	12·13	1·0								
89	5·2	1·4								
90	10·0	5·08								

Both Tubercle Opsonin and Pyocyaneus Opsonin are active at dilutions far higher/

higher than any at which Complement has been known to act. The curve in the case of Tubercle Opsonin is much more even and gradual than in the case of Pyocyaneus where there is a great drop at first and then a much more slow decrease. The latter is somewhat similar to the curve got in two strains of Paratyphoid described by Dr. Fornet and myself. (39)

(4) The Influence of Chemical Reaction.

If Opsonin and Complement were identical or if the opsonic power were dependant on the Complement in any way, we would expect Opsonin to suffer from change in chemical reaction in the same way that the Complement has been shown to do. Ehrlich and Morgenroth (5) found that the presence of 10% Normal Hydrochloric Acid was sufficient to inhibit Complement. Hecker (68) was able to destroy Complement when 0·3cc $\frac{\text{Normal HCL}}{50}$ was added to 0·05cc Serum, that is to say, in the presence of only 1·71% Normal HCL. He was also unable to re-activate Complement by the addition of NaOH. Madsen (15) found that a concentration of 0·003 Normal Hydrochloric Acid in a 1% solution of Bacteriolysin was able to reduce its effect to $\frac{1}{4}$ in the short space of 10 minutes. Noguchi (61) tells us that Amboceptor is comparatively insensitive to HCL. This author finds that the optimum reaction for Opsonin is neutral, for Complement the degree/

degree of alkalinity found in the normal serum. He found Opsonin powerfully affected, however, by an acid reaction, for example, the presence of 3·6% N.HCL. Unlike Hecker (68) he obtained full reactivation of Complement, by the addition of NaOH. He was also able to reactivate Opsonin in the same way.

Exp.	Percentage of Serum Present	Percentage of Normal HCL	Treated Serum Opsonic Count	Count after neutralisation	Control Serum
	80%	5·5	3·5		3·45
44	50%	16·6	0·53		9·0
"	50%	16·6	0·2		10·0
45	80%	0·066	4·5		5·2
"	80%	0·133	5·66		5·2
"	80%	0·66	4·66		"
"	80%	1·33	5·0		"
"	80%	3·33	4·3		"
"	80%	6·66	4·0		"
"	50%	16·6	0·43	(3·0)	"
46	50%	16·6	---	(2·1)	4·0
47	50%	16·6	---	(1·5)	2·5
48	50%	16·6	0·06	(1·0)	3·0
59	50%	16·6	0·05	(0·1)	1·5
"	80%	6·66	1·4		1·5
61	50%	16·6	0·06	(0·5)	2·36
"	80%	6·66	2·0		2·36
"	50%	16·6	---	(0·4)	2·13

61/

Exp.	Percentage of Serum Present	Percentage of Normal HCL	Treated Serum Opsonic Count	Count after neutral-isation	Control Serum
61	80%	6·66	1·5		2·13
		Percentage of Normal Nitric Acid			
59	50%	25	0·16		1·62
	80%	10	0·2		"
	80%	2	1·6		"
	80%	1	1·5		"
	80%	0·5	1·8		"
	80%	0·1	1·65		"

In every instance Opsonin disappeared only with the advent of a precipitate in the serum. The precipitate became later a solid coagulum in the case where Nitric Acid had been used, but not with Hydrochloric. The serum which was neutralised was first allowed to stand in contact with the HCL for half an hour, except in Experiment 59 where different times were tried. Serum which had been treated for half an hour in proportions similar to the above was then diluted with 0·85% NaCL to $\frac{1}{10}$. One cc was then added to 1ccm of $\frac{1}{10}$ Heated Immune Serum, and 1cc 5% sheep's corpuscles. While haemolysis was fully obtained by 0·1cc untreated Complement, 0·1cc Serum which had been treated by 0·066% HCL, gave only a trace of haemolysis. In higher dilutions this/

this was absent, except where so much as 3%--16% HCL had been used there was a partial dark haemolysis from the acid. Only a small part of the Opsonin appears to be recoverable, and in the case of Nitric Acid practically none.

It is, perhaps, interesting in this connection that Hektoen (22) finds when Serum is treated with Oxalic Acid Bacteriolysin is destroyed, while Opsonin is unaffected.

(5) The Influence of Time.

Under this heading we have two questions to consider.

a) Time and the Stability of Opsonin.

R. Pfeiffer (4) has shown that the Complement disappears if the Serum be kept for 8--10 days in vitro. Later, in 1907 Friedberger (69) has studied how long it is possible to keep the Complement, and has found that if diluted and kept in the dark at a low temperature and diluted with water to restore isotonicity it may be kept rather over this time. Wright (9) says that in 3 days the Opsonin has lost $\frac{1}{8}$ or $\frac{1}{2}$ of its value and in a few days longer is inactive. Miss Horton (70) says that if serum be kept in the ice-chest Opsonin will last for three weeks and when in contact with its clot for 32 days[71]. Dean ~~(72)~~, however has discovered Opsonin in the four/

four years old serum of a horse (16). I have many times kept serum sterile not in an ice-chest at all, but merely in a cupboard which often is opened and have found it extremely strong in Opsonin after 21, 23, 25, or 28 days. For example, after 28 days I have found a normal serum give a count of 12 bacteria per leucocyte (Schottmüller's paratyphoid) when 0·85% NaCL gave 0·5 bacteria per leucocyte. In another case also with Schottmüller's strain I have got a count of 19 bacteria per leucocyte in 25 days old normal human serum when the 0·85% NaCL gave 0·6. The same serum was quite non-bacteriolytic. Yet this bacterium is very much more sensitive to bacteriolysis than to phagocytosis. In other cases certainly, a few cases, I have found the serum after 21 days with an opsonic count which was almost nil. In one experience the Opsonin, unlike the Complement which increases for the first few hours in vitro (110, 111)suffered its greatest fall during this time:--

i.e., fresh serum 1·6			same-fresh serum 1·8
	(to Tubercle)		
1 day old " 1·23			same 10 days old 1·3

b) Time and Opsonin Absorption.

Ehrlich and Morgenroth (5) have shown with haemolytic serum that during the first 10 minutes of contact the Amboceptor only has united itself to the cells, after ½ hour the cells begin to dissolve. Böhme (59) is able to get ample phagocytosis in 8 minutes, many bacteria/

bacteria per leucocyte in comparison with inactive serum, although Bacteriolysis was with Normal Serum not appreciable in this time. When it is considered that movements of the leucocytes have to be accounted for within these 8 minutes, the extraordinary vapidity of Opsonin-absorption becomes apparent. Agglutinin and Bacteriolysin are allowed a far longer time to act upon the bacteria before we are satisfied of their presence than is allowed to Opsonin, especially with diluted serum. In one case Opsonin was plainly evident at $\frac{1}{10,000}$ dilution after $\frac{1}{4}$ hour, while after 3 hours at 37°and 24 hours in the ice-chest Complement was able to give only a trace of haemolysis at $\frac{1}{500}$. Not only the final equilibria of Colloid absorptions, but also the velocities are dependant on the chemical nature of the reacting bodies. The presence of Amboceptor on the Bacteria increases and does not hinder Complement-absorption, this is obvious; if, therefore, the rate of Opsonin-absorption without Amboceptor be greater than Complement-absorption with, the two cannot be identical.

Experiment 53/

Length of Incubation

Exp.	Dilution	Normal Human Serum ¼ hour	1-1½hrs.	3½-4 hrs.	5 hrs.	
53	1/1	3·7	5·53	----	2·7	
"	1/50	1·5	2·1	2·62	2·4	Tubercle
54	1/1	2·5	5·7	4·9		
"	1/1	2·42	5·5	3·63		
"	1/1	25·0	38·6			Pyocyaneus

The time-element has, however, a certain importance in Phagocytosis as can be understood when the movements of the leucocytes are considered

The Origin of Opsonin.

It cannot be said that any useful proofs of identity can be made out from the literature on this question. Buchner (1) believes that the Complement is secreted by the polymorphonuclear leucocytes, Metchnikoff(6) holds this too, but thinks that the Ferment ought to act inside the cell and only comes out under unnatural circumstances. Von Baumgarten (74) has not been able to find Complement in the leucocytes. The researches of Bail (29a) appear to support the theory that the Complement originates in the leucocytes, it is now generally held. Löhlein (50) believes that the Opsonin too comes from the same source. Simon, Bispham and Lamar (81) tried/

tried to discover whether there was any relation between the Opsonic power of serums and the leucocyte count, and discovered no relationship. Veitch (75) has proved the same. I have added partially autolysed leucocytes, obtained from an artificial abscess in a dog, and kept sterile under Toluene, to fresh leucocytes and Tubercle bacteria and got a count %0·76, while 0·85% NaCL gave 0·85, and Normal Guinea-pig Serum 3·48. With pyocyaneus bacteria, Normal Guinea-pig Serum gave 0·76, 0·85% NaCL gave 0·3, and Autolysed leucocytes 0·82, Normal Human Serum also gave 0·82. Normal Guinea-pig Serum + Autolysed leucocytes was, however, only 0·76. On the whole the results were not very encouraging to the theory, allowing a margin for error. The same leucocytes contained very powerful ferments of other kinds, proteolytic, diastase etc.

7) Absorption.

It appears that Complement and Opsonin behave in some ways in a similar fashion, and this has led some authors to believe in their identity. Levaditi and Inmann (76), and Neufeld and Hüne (34) have pointed out an analogy in the behaviour of Complement and Opsonin in the presence of yeast cells and various body cells or fragments of cells, both disappear under such treatment. The proteolytic ferment of yeast has been shown harmful to more than ferment, one is Buchner's Zymase (102). It does/

does not follow that Buchner's Zymase and Opsonin are identical. Muir and Martin (82) prove that the presence of a precipitate caused by the union of Antigen with Antibody will absorb Opsonin and at the same time Complement. This is, however, not the only precipitate which will absorb Opsonin. I shall have cause to speak of others, that of the precipitate caused by acid has been already noted. Hedin (112) finds that Trypsin is absorbed by Charcoal, and Simon, Bispham and Lamar (81) that Opsonin is absorbed by Charcoal, but it does not follow that Trypsin and Opsonin are identical. Then again, Muir and Martin (82) have removed both Opsonin and Complement by adding Amboceptor-laden red cells and Amboceptor-laden Bacteria. Ehrlich and Morgenroth (5) have proved that Complement can be washed free of the red cells in the absence of Amboceptor. I do not know of any experiments to prove that Complement can be washed free of bacteria, but could the Complement be fixed directly to the bacteria alone, there would be no need of an Amboceptor, in the sense in which Ehrlich uses the word. Yet Opsonin can undoubtedly be fixed directly to the Bacteria. Bulloch and Atkins (14) have made much of the firmness of this combination which will endure continued washing, also (Wright (9) Bulloch and Atkins (14)) a greater degree of heat than Opsonin/

Opsonin alone. I do not agree with these authors as to the stress which should be laid upon the firmness of this combination because like Dean (16) and K.Meyer (57), I have experienced that a certain amount of the absorbed Opsonin can disappear and as the experiments of Hektoen & Ruediger (19) appear to show. Meyer thinks that Opsonin has been washed away. I am afraid to call it a simple washing-out, because there are well-known disappearances of absorbed substances which are not due to anything so simple, i.,e., the disappearance of HCN in the pores of colloidal Platinum. This disappearance will be discussed later. But that the Opsonin is absorbed is undoubted. If Serum be absorbed with Bacteria at 0° and the Bacteria removed in the cold, the serum contains all the Complement, but all authors agree that ~~the~~ Opsonin, more or less, has disappeared.

Exp.	Normal Human Serum treated with Bact: at 0°	Control Normal Serum	
23	0·16	2·65	
"	0·3	2·65	
33	0·25	1·4	
42	0·05	1·8	
50	1·7	2·85	Tubercle.
51	1·4	2·7	
"	0·9	3·0	
56	0·6	1·44	

Exp.85/

Exp.	Treated	Control	
85	0·4	1·8	
86	0·56	11·3	Pyocyaneus.
91	7·15	12·4	
92	3·5	3·8	

8) The Effect of Dialysis.

The Complement disappears when the serum is dialysed against running water, but Ferrata (79), Brand (80), Hecker (68) have been able to reactivate it again in this way. The removal of the salts in the serum by dialysis causes a precipitate of certain proteids, i.,e..the Englobulins. The Complement which has not dialysed at all has divided into two parts, one of which has precipitated itself down with these proteids, the other exists free in the supernatant fluid. If a portion of the precipitate and of this upper fluid be taken and rendered isotonic, the Complement is again restored. Simon, Lamar and Bispham (81) have also dialysed serum against running water and they have found that the Opsonin precipitates itself down with the Englobulins from which alone it can be restored. They also precipitated the Albumins out of serum by salting at 40°, separated the precipitate carefully, redissolved and dialysed it. The precipitate in the dialysing sac was again dissolved and/

and was found to contain the entire Opsonin, and in its Englobulin fraction. On the other hand Hata (109) believes that he has been able to separate Opsonin by dialysis into two fractions just in the way that Hecker etc. have done for Complement. Yorke (95) who attempted to filter Serum through a porcelain filter stopped up by Gelatine found that Opsonin could be pressed through at first, but that with the formation of a precipitate in the contents of the porcelain tube ceases to come through. He also found by dialysing a sac against 0·85% NaCL no Opsonin appeared in the NaCL. I had decided on this second form of dialysis before I had seen Yorke's paper, because the presence of a proteid precipitate appeared likely to remove the Opsonin before it had much chance of dialysis, if dialyse it could. The fact that Simon, Lamar and Bispham (81) found the Opsonin in dialysed Serum reduced was possibly significant. From other experience in dialysis I was convinced that as small a bore of tube as possible was advisable in order that the rate of diffusion inside the sac itself might not hinder the process. I made, therefore, small collodion sacs able to hold 0·15 to 0·25cc of Serum. I soaked them thoroughly in 0·85% NaCL first placed them in 0·25–03cc NaCL in slightly larger glass tubes, laying them partly on one side and shaking them from time

to/

to time. Now and then at stated intervals I removed samples which were kept separately till I had collected as many as I wished for the experiment. Control Serum was kept under the same conditions as regards evaporation and heat, alongside.

Table	Exp.	Control Normal Human Serum	Dialysed Serum	Length of time dialysed	0·85% Salt Solution Exterior to Sac.	a) Tubercle 0·85% NaCL
	25	3·86	----	1 hour	0·06	0·06
	26b	1·93	1·66	20 mins.	----	----
	"	1·93	1·13	40 "	----	----
	"	1·93	0·93	2½ hrs.	----	----
	27	1·53	0·44	6 days	0·1	0·0
	28	3·46	----	36 hrs.	0·1	0·06
	29	3·5	0·07	6 days	----	0·1
	30	2·16	2·13	¾ hour	----	0·1
	"	2·16	1·26	19 hrs.	----	0·1
	31	1·06	1·13	2½ days	0·0	0·03
					NaCL ext. to Sac	Control NaCL
	95	1·6	1·3	few hrs.	0·03	0·03
	96	1·33	0·6	24 "	----	----
	97	0·8	0·36	2 days	----	----
	98	1·33	1·23	3 "	----	----
	99	2·3	1·5	5 "	----	----
	100	1·6	1·3	2½ "	0·03	0·06

	Exp.	Control Normal Human Serum	Dialysed Serum	Length of time dialysed	NaCL ext. to Sac	Control NaCL
able	34	1·13	0·9	1 hour	----	0·1
	"	1·13	0·96	40 hrs.	----	0·1
	"	1·13	0·4	1 hour	----	0·1
	"	1·13	0·23	40 hrs.	----	0·1
	37	1·96	1·66	20 mins.	----	0·03
	"	1·96	1·13	40 "	----	0·03
	"	1·96	0·93	$2\frac{3}{4}$ hrs.	----	0·03
	39	5·18	4·83	$\frac{1}{4}$ hour	0 13	0·1
	"	----	1·93	$2\frac{1}{2}$ hrs.	0 1	----
	"	----	1·1	4 hrs.	0 1	----
	"	----	0·7	6 "	0 1	----
	"	----	0·33	22 "	0 13	----
	"	----	0·21	30 "	0 06	----
	45	4·8	1·4	2 days	----	----
	46	3·5	1·0	4 "	----	0·6
	"	4·0	1·7	5 hrs.	----	0·6
	47	2·32	0·13	5 days	0 13	0·1
	"	2·5	0·3	1 "	----	0·1
	48	1·8	0·1	7 "	----	0·26
	"	2·5	0·22	3 "	----	0·26
	50	3·26	0·1	5 "		0·2
	77	2·13	0·2	5 "	0 03	0·06

b) Pyocyaneus/

Exp.	Normal Human Serum Control	Dialysed Serum	Length of time dialysed	NaCL ext.to Sac	Control NaCL
	b)Pyocyaneus				
Table 80	14·5	0·86	15 hrs.		----
92	3·8	0·0	1 day		0·0
93	3·33	1·33	1 hour		0·1
"	3·33	0·8	2 hrs.		0·1
"	3·33	0·2	3 "		0·1
	c)Schottmüller's Paratyphoid				
	N.H.S.(Bacteriolysis)	(Bacteriolysis)			
101	5·2 (0)	3·5 (0)	1 hour		3·33
"	5·2	2·1 (∞)	2 hrs.		3·33
"	5·2	1·0 (∞)	3 "		3·33
102	2·43 (0)	2·13 (0)	1 hour		0·13
"	2·43	0·8 (0)	2 hrs.		0·13
"	2·43	0·4 (0)	3 hrs.		0·13
103	1·05 (0)	0·5 (0)	2 hrs.		
"	1·05	0·7 (0)	3 "		
"	1·05	0·3 (0)	4 "		

In Table 101 the Bacteria were allowed to remain in the Control or dialysed Serum for 24 hours before gelatine plates were made, in 102 and 103 for only one hour. Bacteriolysis, however, cannot have been wholly absent in the 2 and 3 hours dialysed Serum in Experiment 101 because/

because the count was less than that of 0·85% NaCL. It is evident from these tables that when Serum is dialysed in this way against a small quantity of NaCL that, 1) no phagocytic power appears in the NaCL outside of the Sac, 2) the Serum inside the Sac gradually loses opsonic power more or less rapidly, more slowly in the case of a reed Sac, at a very varying rate but often very quickly indeed in the case of a collodion Sac. (It is known that collodion films vary very much, it is impossible to make two exactly the same, and here two different samples of collodion were used.) 3) The Bacteriolysin does not necessarily disappear with the Opsonin, but may be present in the absence of the Opsonin. Some haemolytic experiments were undertaken to discover whether the Complement and the Opsonin disappeared together. The results were somewhat equivocal. In most the Complement diminished, in all it was demonstrable. For example, in one case where Control Human Serum and 3 hours dialysed Human Serum were to be compared, 0·25cc of each added to 1ccm each of 5% sheep's corpuscles, showed an equal partial haemolysis, but 0·1cc showed a trace in the case of the Control, and none in the case of the dialysed Serum. The fall in the opsonic power was, 1·96 for the Control, 0·5 for the dialysed. Other cases where Immune Serum was introduced as/

as Amboceptor were done where similar results were obtained. In one case only the Complement disappeared before the Opsonin, in all others the Opsonin more rapidly than the Complement. Under the influence of Time Dilution, and Acid, I have shown the Complement to disappear and the Opsonin to remain, but here we have instances of the disappearance of Opsonin leaving the Complement behind. The argument of Sawtschencko for the Immune body cannot, therefore, be urged in the case of the Complement and the Opsonin, namely, that the Opsonin is the same substance more active in smaller quantities. I have tested Opsonin in respect to these well-known characteristics of the Complement and can find little uniformity between them.

I should like to touch upon two other points upon which I have not myself worked. Sachs and Teruuchi (113) believe that the addition of distilled water will definitely destroy Complement and that it cannot be reactivated by rendering the Serum isotonic afterwards. We are, however, advised to use this very method by Friedberger (69) in order to keep the Complement particularly long. In no other condition will it keep so well. On account of the uncertainty over the behaviour of Complement in this respect, I do not feel called upon to repeat the experiments of Browning (58) which have/

have only just come to my notice. Browning finds Opsonin affected by this treatment in such a way that it cannot be re-activated by making the Serum again isotonic. The treatment is comparable to dialysis against running water and this has already been discussed.

The other point is the deviation of Complement by Dean (18) by the addition of anti-Complement Serum. Can it, however, be proved that an anti-Complement Serum does not also contain anti-Opsonin? Again, it must be remembered Serum precipitates are apt to absorb Opsonin. I can find nothing but a few arguments from analogy to lend weight to the view that Complement and Opsonin are identical. On account of many points of difference which I have met with I am forced to believe, not only that these substances cannot be identical, but also, that Complement can have no part in opsonic action.

B. Normal Opsonin and Amboceptor.

Neufeld and Hüne (35), and also Bächer (45) have met with cases of serum full of Amboceptor but devoid of Opsonin. Barratt (53) and Keith (52) and Neufeld and Bickel (36) have managed to separate Haemolytic Amboceptor and Haemo-opsonin or Haemotropin. Sawtschencko (37), Dean (16), and also Levaditi at one time have believed that Immune Opsonin is identical with bacteriolytic Amboceptor. It is not yet determined whether or not Normal Opsonin is identical with Immune Opsonin. Dean (16) believes that they are identical. On this account it is necessary to consider the possibility of the identity of Normal Opsonin with Amboceptor.

Amboceptor is recognised by four characteristics, 1) stability, 2) comparative insensitiveness to chemical reaction, 3) specificity, 4) absorption properties.

1) Stability:---

Keith (52) has proved that haemolytic Amboceptor is practically unaffected by heating at 55° for half-an-hour, while haemo-opsonin is much affected. Bordet (2) has shown that bacteriolytic Amboceptor can endure a temperature of 56°-60° for half-an-hour. We have shown that opsonin is much affected at 51°. Amboceptor will keep for many months without apparently suffering, while opsonin has already lost strength in twenty-four hours.

Opsonin/

Opsonin appears indeed to stand between Amboceptor and Complement, to be more labile than Amboceptor and more stable than Complement.

2) Chemical Reaction:---

Amboceptor appears to be comparatively insensitive to acid. Noguchi (61) finds that in an acid reaction so great that Opsonin Absorption does not take place at all, Amboceptor is fully absorbed and fixed.

3) Specificity:---

Various authors have proved that Immune Opsonin is specific, Leishman(8), Schottmüller and Much (93) and others. Bulloch and Western (13) have absorbed Normal Serum with Bacteria of one kind and find that more Opsonin is lost to the particular organism than to others. Yorke and Smith (94) have repeated Bulloch and Western's (13) experiments with Normal Opsonin and disagree with them. They find Opsonin to all kinds of bacteria affected when Serum has been absorbed with one. This does not appear a very clear proof against specificity, on account of mechanical surface attraction other Opsonins than the specific may be centrifuged down with the bacteria and lost in separation. Simon Lamar and Bispham (81) have noticed the phagocytic power of a serum towards two different kinds of bacteria disappear at the same dilution. Fornet and I have carried opsonin to one strain of typhoid only to $\frac{1}{1000}$, to other strains to $\frac{1}{10,000}$. After heating to 56° Normal Human/

Human Serum is still actively phagocytic to pyocyaneus while at 51° Opsonin to Tubercle is much affected. This may be explained by supposing that the amount of Opsonin necessary for different bacteria varies, rather than the quality. It appears, however, probable that Opsonin is, like Amboceptor, really specific.

4) Absorption:---

Ehrlich and Morgenroth (5) have shown that the Amboceptor is absorbed by red cells at 37° and that this also occurs at 0°-3°, that all the Amboceptor goes into the union, that the union is firm and cannot be undone by continued washing. At 0° Hektoen and Ruediger find that the rate of Opsonin-absorption is very greatly reduced. Löhlein and Bulloch and Atkin (14) could find no Opsonin left in serum which had been treated at 0°. They also believed that the bacteria were at 0° perfectly prepared for phagocytosis. Unfortunately the method of procedure of these earlier authors has not been so exactly detailed as to make it easy to faithfully repeat their experiments. Other and later writers, Dean (16), Meyer (57), Cowie and Chapin (55), Fornet and I (39), have found that bacteria treated with serum at 0° are not ready for phagocytosis and are either not opsonised at all or only very slightly. The methods adopted by most of these later authors have been satisfactory as far as cooling both serum and bacterial emulsion before mixing and later centrifuging in

the/

the cold. To this day, however, there is doubt as to the extent to which Opsonin disappears out of serum which has been so treated, and its significance. Is it that the Opsonin is absorbed,but not firmly, so that it can again be washed out? K. Meyer (57) reports that Bacteria which have been treated with serum at 37° can lose a part of their Opsonin. Dean (16) says that Bacteria may take up more Opsonin than they require and later give it off to the fluid in which they are washed. Hektoen and Ruediger (19) give figures which show that a half, or almost a half, of the Opsonin is lost in this way. Or, is it,as Meyer (57) and Cowie and Chapin (55) believe, that at 0° only an Amboceptor is absorbed, so that both treated Bacteria and treated serum are inactive, the one requiring the other to be re-activated. These discrepancies, or rather uncertainties, as to whether the Bacteria are fully opsonised at 0° or not, or as to whether any part of the Opsonin is absorbed at 0°, and as to the absolute amount of Opsonin absorbed, can in part be explained by certain physical laws. It was found out by Zawidski (83) as early as 1900, and has been later confirmed by other physical chemists, that the concentration of a solute differs, it may be as much as several per cent in the surface layer from the body of a solution, either less or more. In the latter case, any increase of the surface will necessarily diminish the amount of the solute in the body of the/

the solution. The addition of any insoluble material to the solution will increase the surface. In the case of Opsonin we do not know whether or not it exists in true solution, probably not, on account of the fact that it will dilute so far. But the effect of surface tension is not only exerted on dissolved substances but most obviously also on suspended particles. Insoluble particles thrown into a fluid will partly remain buoyed up by the surface although gravitating rapidly once they have passed below it, if a bacterial emulsion, which is so well known to contain clumps in the surface layer that the purchaser is warned of the fact on certain commercial samples of Cultures, be left to settle, the Bacteria settle upon the sides all the way down as well as on the bottom, although to a less extent. Following this simple rule, we might expect it possible that the Opsonin as well as other constituents of the serum would be deposited mechanically on the surface of the Bacteria, or to be at least increased in their neighbourhood. But in the case of Bacteria another law applies, we are here dealing with a surface which is also a porous colloidal membrane capable of acting as a solid solvent. Now, where two liquid solvents are in contact a solute is divided between them in a certain definite proportion which is a simple constant, say that with a certain concentration the proportion of 1 to 5 exists, it exists also at all other concentrations. But

where a liquid and a solid solution are in contact, the distribution of the solute in each is expressed by a formula in which the total concentration is a factor $\frac{C_1^n}{C_2} = K$ The figure "n" may be a minus quantity, it may be as much as +6, depending on the chemical nature of the reacting substances. If, for example, "n"=2, and with a certain concentration the distribution of the dissolved substance was 4 in the solid to 2 in the liquid system and 30 more parts were added, they would divide as 25:5, not as 20:10. If 72 parts were added, as 64:8 and so on. If we are dealing with a case of solid solution in Opsonin-absorption then the more concentrated the Opsonin the greater the difference between the absorbed and the free, the more dilute the converse. It would be interesting to discover whether by diluting the treated serum less Opsonin proportionately would be lost.

Exp.	Untreated Bacteria + 2 parts 0·85% NaCL.	Bacteria treated with Normal Human Serum at 0° + 2 parts 0·85% NaCL.	Normal H.S. undiluted + untreated Bacteria + 0·85% NaCL.	Undiluted Normal H.S. treated with Bacteria at 0° + untreated Bacteria + 0·85% NaCL.	
23	0·1	0·1	2.65	0·16 0·3	Tubercle
33	0·13	0·85	1·4	0·25	
49	0·1	0·0	2·94	----	
50	0·2	0·1	2·85	1.7	
51	0·08	0·0	2.7	1·6	
56	0.0	0.0	1·44	0.6	
77	0·06	0·05	1·98	0.3	

Undiluted.

Exp.	Untreated Bacteria	Treated Bacteria	Normal H.S. + Untreated Bacteria	Serum treated with Bacteria at 0° + Untreated Bacteria.	
85	0.1	0.3	1.8	0.4	Pyocyaneus
86	0.4	0.26	11.3	0.56	
87	0.3	0.35	1.1	0.6	

Diluted

Exp.	Untreated Bacteria	Treated Bacteria	N.H.S.dilut- ed + untreat- ed Bacteria	Diluted Serum treated at 0° + Untreated Bacteria	
41	0.18	0.05	5.7 $(\frac{1}{5})$	--- $(\frac{1}{5})$	Tubercle
42	0.1	0.02	0.06 $(\frac{1}{5})$	0.08 $(\frac{1}{5})$	
79	0.0	0.0	0.82	0.75	
91	0.83	1.05	8.0 $(\frac{1}{4})$	7.15 $(\frac{1}{4})$	Pyocyaneus
92	0.0	0.12	4.1 $(\frac{1}{4})$	3.8 $(\frac{1}{4})$	

It appears that concentrated serum loses more of its Opsonin than diluted serum. Several instances bearing out the fact that diluted serum loses very little Opsonin may be seen in Fornet and Porter (39). Both, however, lose it according to these tables to some extent, more or less, this shows that some of the Opsonin has gone into the Bacteria undoubtedly. It is remarkable that if the serum-bacteria is not centrifuged in ice, the bacteria are ready for phagocytosis if leucocytes are added, while if the serum be separated from the Bacteria in the cold this is not the case. There are two explanations of this, 1) that a Complement in the serum has been enabled to act by the rising/

rising temperature, 2) that absorption, or rather surface attraction of Opsinin, the first stage of Opsonation, takes place at 0°, but not the second stage of fixation and union. Which is the true explanation is easy to discover and will be dealt with under the head of Re-activation later. The discrepancy, however, between those authors who centrifuged in ice and centrifuged also their washed bacteria in ice and did not find the bacteria opsonised, and those authors who do not say that they took these precautions and discovered the bacteria quite ready at the end of their treatment for phagocytosis, becomes better explained. We have also explained why the amount of Opsonin left in treated serum varies, 1) by the number of bacteria causing varying extent of surface, 2) by varying concentration of Opsonin.

It is evident that at 0° the Opsonin (apart from a possible factor of it) is not, like the Amboceptor, firmly fixed to the Bacteria. It cannot, therefore, be identical with the Amboceptor. Also, because all the Opsonin does not disappear out of the Treated Serum if precautions are taken, as all the Amboceptor does, the two cannot be the same.

It would be interesting to discover whether the Opsonin be fully absorbed at 37° and whether this Opsonin is so firmly fixed that it cannot be washed away. Bulloch and/

and Atkin (14) and Hektoen and Ruediger have absorbed bacteria with serum at 37° and washed and have obtained an opsonic count, high, but always less than when serum and leucocytes were added to the bacteria together. This has been ascribed to loss of bacteria by washing but surely the two emulsions were made up to the same standard. Dean (16) has found that when bacteria which have been treated with Immune Serum are washed, Opsonin is washed off to a certain extent. K.Meyer(57) has treated Bacteria with Serum at 37° and finds the count very much lower than that where untreated Bacteria Serum and Leucocytes are mixed together at once. Fornet and I (39) have found the count much lower too, in the case of Paratyphoid. It was, however, higher than when Bacteria were treated at 0°. I have absorbed Bacteria with Serum at 37° and at 0° and also heated Serum 56° at 37° with the following results:---

Exp.	Normal Serum+ Untreated Bacteria.	NaCL+Untreated Bacteria.	NaCL+Bacteria treated 0° with Fresh Serum.	Bacteria treated 37° with Fresh Serum.	Serum treated with Bacteria at 37°.	Bacteria treated with 56° heated Serum at 37°.
82	3·6	0·1	---	0·5	(0·53)	0·2
83	0.83	0·03	---	0·23	(0·3)	0·1
86	11·3	0·4	0·26	1·26	(0·8)	0·7
87	1·1	0·3	0·35	1·0		0.3
91	5·16	0·83	1·05	3·93	(1·55)	---

In these instances the Opsonin has been better absorbed at 37°/

37° than at 0°, a part is actually fixed to the bacteria, but a great part has been lost by washing or in some other way. In its absorption-properties, then, the Opsonin appears to stand midway between the Complement and the Opsonin, it seems to be very much better absorbed than the Complement and yet not so well fixed as the Amboceptor. It is necessary, however, to complete these absorption experiments by adding Complement to the treated Bacteria and this we shall do under the next section.

C. I wish next to consider whether Normal Opsonin is of simple structure, or if it has an Amboceptor-Complement Form. This view is held by Neufeld and Hüne (34), Cowie and Chapin (55), Dean (18), K.Meyer (57), Haetjens (109), and Caulwell (56), for reasons which have already been discussed.

the latter ?

Re-activation Experiments. a) Tubercle.

Exp.	Normal Guinea-pig Serum.	Normal Guinea-pig Serum heated 60°.	Fresh + Heated.
5	3·4	0.96	3·6
6	5·5	0.94	1·28
9	2.8	0.78	0.7
65	1·6	0·26	1·0
66	1.62	0·2	0.4

Exp.	Normal Human Serum + NaCL.	N.H.S. heated 56°+NaCL.	N.H.S. heated 60°+NaCL.	Fresh+ heated 56°.	Fresh+ heated 60°
10	2.03	0·2	0.55	1·0	2·36
11	3.85	0·23	0·63	1.22	3.8
12	3·45	0·62	0·12	3·4	3·7
13	2·85	0·23	0·2	2·2	2·6
14	2·66	0·3	0·23	1·33	---
15	4.2	0·5	---	2.06	---
20	3.5	0·4	0·2	1·4	3.36
"	3·56	0·23	0·33	2·7	3·4

21/

Exp.	Normal Human Serum +NaCL.	N.H.S. heated 56° +NaCL	N.H.S. heated 60° +NaCL.	Fresh+ heated 56°.	Fresh+ heated 60°.
21	4·36	0·5		3·73	
"	5·0	0·4		4·23	
22	1·16	0·2		0·7	
23	2·65	0·13		1·2	
24	3·5	0·35		2·8	
26	1·93	----		0·86	
32	1·83	----		1·26	
36	2·8	----		1·5	
37	1·96	----		0·86	
44	10·0	0·8		7·0	
46	4·0	1·0	1·0	3·5	3·9
47	2·5	0·2		2·2	
48	3·0	0·3		2·9	
50	2·85	0·2		2·06	
51	2·7	0·2		2·56	
54	2·5	0·2		2·08	
55	1·86	0·16		1·5	
"	1·36	0·1		1·2	
57	1·56	0·4		1·5	
64	4·09	0·2		2·75	
65	1·54	0·52	0·26		1·06
66	1·2	0·06		0·5	
67/					

Exp.	Normal Human Serum + NaCL.	N.H.S. heated 56° NaCL.	N.H.S. heated 60° NaCL.	Fresh+ heated 56°.	Fresh+ heated 60°.
67	1·27	0·06		0·66	
"	0·9	0·1		1·02	
69	0·82	0·2		0·7	
70	0·7	0·03		0·72	
"	0·7	0·03		0·66	
74	1·5	0·25		1·2	
75	1·3	0·08	0·1	0·93	0·26
77	2·7	0·15		2·06	
	b) Pyocyaneus.				
83	0·83	0·2		0·8	
84	1·6	---	0·6		0·9
"	----	---	0·4		0·8
"	----	---	0·6		0·45
"	----	---	0·43		0·3
85	1·8	0·4		2·1	
86	1·1	0·6		1·23	
94	3·8	0·3		2·0	

No single case of re-activation can be seen in this whole table. Instead of this, an antagonism is apparent between heated and unheated Serum in nearly every case. If 2 parts of Normal Serum are added together instead of Normal Serum + 0·85% Salt Solution the count appears to be raised, but not doubled.

Exp./

Exp.	Human Serum 1 part + NaCL.	Human Serum 2 parts.
15	4·76	6·4
16	4·76	5·33
17	4·2	4·43
18	5·6	5·93
9	1·08	2·2
86	11·3	16·0
	Guinea-pig Serum 1 part + NaCL.	Guinea-pig Serum 2 parts.
9	2·7	4·5
"	5·83	7·1

It may be, however, that I have not given a good enough chance for the re-activation to be shown by using undiluted Serum as Complement. The ideal conditions for a Re-activation Test are where one can build up from two noughts a decided positive result, such as one may obtain in the case of Haemolysis and Bacteriolysis. Unfortunately, Tubercle-Opsonin is less affected by dilution than many other Opsonins, still, by using a very dilute bacterial emulsion it is possible to obtain a very low figure indeed for Serum which is not too far diluted.

Exp.	Dilution	Normal Guinea-pig Serum + NaCL.	(Undiluted) N.G.S. heated 60° + NaCL.	Heated + Diluted Fresh
1	$\frac{1}{10}$	2·42	0·4	1·4
"	$\frac{1}{50}$	0·6	"	1·2
"	$\frac{1}{100}$	1·1	"	1·1

Exp.	Dilution	Normal Guinea-pig Serum + NaCL.	(Undiluted) N.G.S. heated 60° + NaCL.	Heated + Diluted Fresh
3	1/10	2·2	0·54	1·6
"	1/50	1·9	"	1·0
"	1/100	1·3	"	1·4
4	1/10	0·96	0·5	0·57
"	1/50	0·8		0·62
"	1/100	0·7		0·54
5	1/10	2·64	0·96	1·68
"	1/50	1·96		1·36
"	1/100	1·5		1·18
6	1/10	3·24	0·94	1·08
"	1/50	2·6		0·92
"	1/100	1·6	N.G.S. 56° ... Heated + Fresh, 56	0·9
8	1/100	0·78 (1·34)	0·78 (1·56)	0·76
65	1/20	0·32	0·26	0·1
66	1/20	0·26	0·2	0·13

Dilution		Normal Rabbit Serum + NaCL.	N.R.S. heated 56° + NaCL	N.R.S. 60° + NaCL	Fresh + 56° heated	Fresh + 60° heated.
62	1/12	0·7	0·16	0·06	0·5	0·6
63	1/12	2·3	0·9	0·2	2·1	1·9

Exp.	Dilution	Normal Human Serum + NaCL.	N.H.S. heated 56° + NaCL	N.H.S. heated 60° + NaCL.	Fresh + 56° heated	Fresh + 60° heated
14	1/10	0·4	0·3		0·33	
15	1/10	2·0	0·7		2·66	

16/

Exp.	Dilution	Normal Human Serum + NaCL.	N H S. N.H.S. heated 56° + NaCL	N H S. heated 60° NaCL	Fresh + 56° heated	Fresh + 60° heated
16	1/10	2·1	0·53		1·7	
17	1/10	2·5	0·5		1·23	
57	1/20	0·8	0·4		0·68	
58	1/20	0·54	0·06		0·68	
60	1/20	0·6	0·3		0·8	
67	1/20	0·2	0·6		0·14	
"	"	0·2	0·1		0·2	
69	1/20	0·08	0·2		0·3	
"	"	0·08	0·06		0·05	
70	1/20	0·2	0·03		0·0	
"	"	0·2	0·03		0·03	
71	1/20	0·36	0·1		0·23	
72	1/20	0·3	0·1		0·03	
"	"	0·3	0·12		0·16	
73	1/20	0·22	0·0		0·03	
"	"	0·22	0·18		0·06	
74	1/20	0·4	0·25		0·4	
"	"	0·4	0·25		0·65	

There are in these tables only three instances where Diluted Complement + heated Serum gave a count greater than their sum, and the increase is 0·2 on 1·0, 0·08 upon 0·6, and 0·02 upon 0·28, all three being so slight as to be within the margin of error. The other instances show generally/

generally an antagonism, occasionally indifference. This antagonism reminds one forcibly of the inhibition produced by Cramer and Bearn (84,85), Pollack(86), Schwarz (87), by adding solutions of heated ferments to solutions of fresh ferments, this antagonism varying in extent, being sometimes absent. Rarely an actual acceleration occurred. Like the antagonism of heated ferment, that of heated Serum comes up in different specimens at different temperatures. In cases where the antagonism was less marked at lower temperatures it was obtainable at higher, both with Diluted and Undiluted Serum.

			Normal Human Serum heated				
Exp.	Dilution	Normal Human Serum +NaCL.	58° +NaCL	60° +NaCL	65° +NaCL	70° +NaCL	Fresh + Heated
46	1/1	4·0	0·93				3·06
"	"	4.0		1·0			3·9
68	"	0·75			0·03		0·45
"	"	0·75			0·02		0·1
69	"	0·82			0·03		0·43
71	"	1·55			0·03		0·7
"	"	1·55				0·03	1·37
73	"	1·23			0·03		0·26
74	"	1·5			0·03		0·76
68	1/20	0·12			0·03		0·1
"	"	0·12			0·02		0·05
71	1/20	0·36			0·03		0·13
"	"	0·36			0·12		0·0

72/

Exp.	Dilution	Normal Human Serum + NaCL.	Normal Human Serum heated 58° + NaCL	60° + NaCL.	65° + NaCL	70° + NaCL.	Fresh + Heated
72	1/20	0·3		0·03			0·0
"	"	0·3					0·0
73	1/20	0·22					0·0
"	"	0·22					0·1
74	1/20	0·4					0·3

It will be remembered that pyocyaneus Opsonin lost at first rapidly by dilution, such dilutions as one-fourth reducing the count very much. One would imagine in such a case that if Re-activation could occur it should be very marked.

Pyocyaneus Opsonin

Exp.	Dilution	NaCL + Normal Human Serum	NaCL + N.H.S. heated 56°	NaCL + N.H.S. heated 60°	Fresh + heated 56°	Fresh + heated 60°
80	¼	3·3	6·46		4·6	
81	¼	1·0	0·4		1·36	
"	¼	1·0	0·2		0·93	
"	¼	1·0	0·93	0·2	0·55	0·6
"	"	1·0	0·9		0·9	
"	"	1·0		0·2	---	0·35
82	"	0·6	0·6		0·4	

84/

Exp.	Dilution	NaCL + Normal Human Serum	NaCL + N.H.S. heated 56°	NaCL + N.H.S. heated 60°	Fresh + heated 56°	Fresh + heated 60°
84	¼	1·0	0·43	0·4		0·5
"	"			0·6	1·4	
"	"			0·43	0·55	
"	"			0·6	1·2	
85	"	0·35	0·4		0·4	
86	"	0·5	0·8		0·06	
87	"	0·5	0·6		0·4	
88	"	1·0	2·15		1·1	
"	"	1·0	1·73	1·43	0·8	0·4
"	"	1·0	1·73	1·3	0·5	1·6
"	"	1·0	2·8	2·4	1·76	1·43
"	"	1·0		3·6		2·0
89	"	1·4	1·7	0·8	3·6	0·2
"	"	1·4	2·1	1·2	3·74	1·55
"	"	1·4	1·63	1·43	1·64	2·4
"	"	1·4	1·2	1·45	2·66	1·4
90	"	5·08	2·85	2·8	2·5	3·2
"	"	5·08	2·3	2·08	2·3	2·0
"	"	5·08	2·33	2·5	3·6	3·0
"	"	5·08	2·0	---	1·9	---
"	"	5·08	2·8	2·05	1·2	3·0
"	"	5.08	3.4	2·0	1·2	---

There is here no evidence of Reactivation whatever.

Serum/

Serum heated at 56° for half an hour and Serum treated with Bacteria at 0° are both inactive, bacteriolytically, the one containing only Amboceptor, the other only Complement. Serum heated or treated in this way has been shown to lose a certain part of its opsonic power. It would be interesting to discover whether Reactivation is possible by mixing the two.

Exp.	Normal Human Serum heated 56° + NaCL	N.H.S. treated with Bacteria at 0° + NaCL	Heated + Treated Serum	
23	0·13	0·16	0·7	
50	0·2	1·4	1·6	
51	0·2	1·4	1·13	Tubercle
		0·9	0·12	
77	0·15	0·3	0·2	
106	0·52	0·98	0·4	
85	0·4	0·4	0·5	
86	0·8	0·56	0·36	Pyocyaneus
92	0·3	3·5	1·2	
105	2·6	5·0	2·5	
107	6·0	4·25	5·1	Paratyphoid
108	3·53	5·5	4·62	

There is only one instance here where the count of heated + Treated Serum mixed is greater than the sum of their counts alone. In this case untreated Normal Serum gave a count of 2·65/

2·65, so that 0·7 does not appear a very satisfactory re-activation, especially as the other instances do not support this explanation. In the last three experiments with Paratyphoid, there was successful re-activation in corresponding bacteriolytic experiments.

Attempt to re-activate Dialysed Serum.

Although Opsonin may have nothing to do with either the Amboceptor or the Complement, still it may possess two interdependent constituents. I have, therefore, made one or two experiments which are not justifiable from the standpoint of whether Opsonin and the bacteriolytic Amboceptor and Complement are identical, but which come best under the section of Re-activation, and which I dare not omit on account of the fact that through them I made an interesting discovery.

Magnus (88) found that when liver extract was dialysed the fat-splitting ferment disappeared, but could again be re-activated by the addition either of a portion of the dialysate or of the original extract inactivated by heat. Harden and Young (89) proved the same for yeast juice. Although the cases are not similar, because these ferments were dialysed against running water and inactivation was due to loss of certain electrolytes, I have attempted to re-activate dialysed Serum in various ways.

1) Dialysed Serum + NaCL solution outside the dialysing sac.

Dialysed/

Exp.	Dialysed Serum + 0·85% NaCL.	NaCL outside the Sac + 0·85% NaCL	Dialysed Serum + NaCL outside the Sac
39	0·33 (22 hrs.)	0·13	0·25
	0·21 (30 ")	0·06	0·3
40	0·4 (2 days)	----	0·23
47	0·13 (5 ")	0·13	0·26
77	0·2 (5 ")	0·03	0·13

2) Dialysed Serum + Serum heated 56°.

Exp.	Dialysed Serum + NaCL	N.H.S.heated 56° + NaCL.	Dialysed Serum + N.H.S.heated 56°.	
77	0·2 (5 hrs.)	0·15	0·25	Tubercle
80	0·86(15 ")	6·46	1·55	Pyocyaneus
92	0·0 (3 ")	0·3	0·3	
101	1·0 (3 ")	0·6	1·2	Paratyphoid

3) Dialysed Serum + fresh unheated Serum.

Exp.	Fresh Normal Serum + NaCL	Dialysed Serum + NaCL.	Fresh + Dialysed Serum.
26	----		
35	1·03	---- (20 hrs.)	0·7
39	5·18	0·21 (30 hrs.)	4·9
40	2·98	0·4 (2 days)	2·7
46	4·0	1·0 (4 ")	2·75
47	2·5	0·13 (5 ")	1·92
48	3·0	0·1 (7 ")	3·2
"	3·0	0·22 (3 ")	2·9
49/		---- (")	
		---- (")	

Exp.	Fresh Normal Serum + NaCL	Dialysed Serum + NaCL	Fresh + Dialysed Serum
49	2·94	---- (8 days)	1·9
"	2·94	---- (4 ")	2·2
50	2·85	0·1 (5 ")	1·96
77	2·7	0·2 (5 ")	2·5
Pyocyaneus			
80	14·5	0·86 (15 hrs.)	0·9
92	3·8	0·0 (3 ")	3·5
Paratyphoid			
101	5·2	1·0 (3 hrs.)	5·4

There is only one instance here where Fresh + Dialysed Serum gave a higher count together than the sum of their separate counts, and this increase is so slight as to be within the margin of error. The other instances seem to point to a distinct antagonism. Not only does Opsonin disappear in dialysed Serum and cannot be re-activated in the ways attempted, but actually an antagonistic substance is produced in its place. Anti-ferments differ in their behaviour to dialysis. Hamill (90) found that the Anti-trypsin of Weinland will dialyse through Collodion. Bearn and Cramer (85) report the same of the antagonistic "zymoid" of pepsin. Röden (91) says that anti rennin in Serum will not dialyse. It would be interesting to find whether the salt solution in contact with the dialysing sac developes any antagonistic qualities, in the case of Opsonin.

Fresh/

Exp.	Fresh Human Serum + 0·85% NaCL	NaCL from outside the Sac + untreated 0·85% NaCL		Fresh Serum + NaCL outside the Sac
25	3·86	0·06	(1 hour)	3·2
26	2·66	----	(2 days)	1·9
28	3·46	0·1	(36 hrs.)	1·33
34	1·13	----	(20 mins.)	0·4
"	1·13	----	(20 ")	0·16
"	1·13	----	(40 ")	0·2
"	1·13	----	(40 ")	0·43
"	1·13	----	(40 ")	0·23
"	1·13	----	(1 hour)	0·16
"	1·13	----	(1 ")	0·43
31	0·8	0·0	($2\frac{1}{2}$ days)	0·36
39	5·18	0·13	($\frac{1}{4}$ hour)	4·98
"	5·18	0·1	($2\frac{1}{2}$ hrs.)	3·5
"	5·18	0·1	(4 ")	3·02
"	5·18	0·1	(6 ")	2·7
"	5·18	0·13	(22 ")	2·75
"	5·18	0·06	(30 ")	2·5
40	2·98	0·2	(2 days)	0·7
47	2·5	----	(5 ")	0·26
95	1·6	0·03	(few hrs.)	1·0
96	1·33	----	(24 ")	0·66
97	0·8	----	(2 days)	0·3
98	2·3	----	(5 ")	1·13
99	1·6	0·03	($2\frac{1}{2}$ ")	0·96

It/

It is very evident that an antagonistic substance is able to dialyse through the membrane, and to do so often rapidly. The permeability of Collodion varies very greatly, and it is almost impossible to make two Collodion Sacs the same. Hektoen (22) says that Alcohol is anti-opsonic.

In order to make certain that the action of the Collodion was not due to Alcohol given off by it, although in each case the Sac when made was soaked in much water and later in 0·85% NaCL before use, the following experiment was undertaken. A Sac was made and soaked in water and 0·85% NaCL as usual and then cut in two, the upper part placed in 0·2cc NaCL (0·85%) and 0·2cc Serum put in the lower part and dialysed for 2½ hours. At the end of that time, Control Serum + untreated NaCL gave 1·93 bacteria per leucocyte, while Control Serum + NaCL from the Collodion 1·96. The dialysed Serum + untreated 0·85% NaCL gave 0·73.

Attempt to re-activate old Serum.

Unfortunately the time during which Opsonin remains inactive is somewhat variable. I have only in two instances succeeded in obtaining three weeks old Serum which had almost entirely lost its Opsonin to Paratyphoid.

Fresh/

Fresh Human Serum $\frac{1}{4}$	3 Weeks old H.S.	Fresh + Old
0·8	0·3	1·0
0·2	0·02	0·2

Three weeks old Serum is inactive bacteriolytically. I have, however, been able to re-activate it against Schottmüller's Paratyphoid, by adding 0·5cc old Serum to 0·5cc Serum treated with Bacteria at 0^{o} + 0·025cc of an emulsion of Paratyphoid which gave with leucocytes an opsonic count of 7·13 for old Serum, 4·25 for treated 0^{o} Serum and 4·0 for both mixed.

Absorption/

Absorption Experiment on the effect of adding Complement to Amboceptor-laden Bacteria

Exp.	Untreated Bacteria + 0·85% NaCL	Bacteria treated with Serum at 0° + NaCL	Bacteria treated with Serum at 37° + NaCL	Bacteria treated with 56° Serum + NaCL	Untreated Bacteria + N.H.S.	Bacteria treated 0° + N.H.S.	Bacteria treated 37° + N.H.S.	Bacteria treated with heated Serum at 37° + N.H.S.
23	0·1	0·1	----	----	$(\frac{1}{1})$ 2·65	0·4	----	----
26a	0·13	---	----	0·6	" 2·8	----	----	1 86
36	0·06	---	----	0·06	" 2·8	----	----	1 85
41	0·18	0·05	0·05	----	8·93	1·42	5·0	----
"	0·18	0·05	0·05	----	$(\frac{1}{5})$ 5·7	0·7	2·2	----
42	0·1	0·02	----	----	1·8	0·1	----	----
"	----	----	----	----	$(\frac{1}{5})$ 0·06	0·1	----	----
44	0·46	----	----	0·36	10·0		----	5 86
49	0·1	0·0	----	----	2·94	2·3	----	----

Tu-
bercle

50/

Exp.	Untreated Bacteria	Bacteria treated 0°	Bacteria treated 37°	Bacteria treated with heated 56° Serum at 37°	Untreated Bacteria + N.H.S.	Bacteria treated 0°+N.H.S.	Bacteria treated 37°+N.H.S.	Bacteria treated with heated Serum at 37° + N.H.S.	
50	0·2	0·2	----	----	2·85	2·45	----	----	
51	0·08	0·0	----	----	2·7	1·6	----	----	Tubercle
56	0·0	0·0	----	----	1·44	1·0	----	----	
42	0·3	1·1	----	----	0·6	2·8	----	----	Typhoid
82	0·1	----	0·5	0·2	(¼)0·6	----	0·25	0·5	
83	----	----	0·23	0·1	0·83	----	0·64	0·85	
85	0·1	0·3	----	----	1·8	1·3	----	----	
86	0·4	0·26	1·26	0·7	11·3	9·6	----	6·2	
"	0·4	0·26	1·26	0·7	(¼)0·5	0·53	1·66	0·8	Pyocyaneus
87	0·3	0·35	1·0	0·3	1·1	1·1	1·3	1·2	
"	----	----	----	----	(¼)0·5	0·45	1·1	0·4	
91	0·83	1·05	3·93		(¼)5·16	3·6	2·26	3·5	

42/

Exp.	Untreated Bacteria	Bacteria treated 0°.	Bacteria treated 37°	Bacteria treated with heated 56° Serum at 37°	Untreated Bact.+Serum treated with Bact. 0°.	0° Treated Bacteria+ Treated Serum	37° treated Bacteria + Treated Serum	Bacteria treated with heated 56° Serum + Treated Serum	
42	0·1	0·02	----	----	$\left(\frac{1}{5}\right)$ 0·05	0·1	----	----	
51	0·08	0·0	----	----	0·98	0·88	----	----	Tubercle
106	0·1	0·2	----	----	1·4	0·93	----	----	
42	0·3	1·1	----	----	$\left(\frac{1}{5}\right)$ 0·1	0·0	----	----	Typhoid
85	0·1	0·3	----	----	$\left(\frac{1}{4}\right)$ 0·4	0·5	----	----	Pyocyaneus
86	0·4	0·26	1 26	0 7	$\left(\frac{1}{4}\right)$ 0·56	0·6	----	0·65	

There/

There is only one case where a Re-activation occurs and this is in the case of typhoid Bacteria treated at 0° with Serum and supplied later with Fresh Serum Complement. The result is, however, negatived by the result obtained by adding Serum which has been treated at 0° with Bacteria and must, therefore, contain more free Complement than the Fresh Serum, to the very same Bacteria where the count is actually lowered. I have tried to control these results by testing the treated Serum for Complement.

Normal/

		Opsonic Count	Bacteriolysis
Normal Human Serum	+ 0·85% NaCL	9·05, 8·4	0·5cc of each + 0·025cc Bacterial emulsion = 0
N.H.S. heated 56° "	+ "	2·6, 6·0	" " " = ∞
N.H.S. treated with Bact. at 0°	+ "	2·0, 4·25	" " = ∞
Bacteria treated with Serum at 0°	+ " 2 parts	2·0, 1·3	" " = ∞
Untreated Bacteria	+ " "	0·62, 1·2	" " = ∞
N.H.S. heated 56° + N.H.S. treated at 0°		2·5, 5·1	" " = 0
N.H.S. treated at 0° + Bacteria treated at 0°		2·1, 2·7	" " = 0

Exp. 105 106 (Incubation 1 hour.)

There/

There is no choice for me but to refuse the theory that Opsonin has an Amboceptor-Complement form. From these results it appears to me as impossible as that the Opsonin and the Amboceptor, or the Opsonin and the Complement, should be identical. The Opsonin is a body of simple structure, new, having no other function, it is more easily absorbed by the Bacteria than the Complement, less firmly fixed than the Amboceptor, more stable than the Complement, less stable than the Amboceptor, and able to be antagonised by heated Serum. Like the antagonism exerted by heated ferment solutions described by Bearn and Cramer (84 & 85), Pollack (86) and Schwartz (87), by precipitin serums described by P.T. Müller (98) Welsh and Chapman (96 & 97), this antagonism is very variable, varying in different specimens, not only in strength but also in the temperature necessary to call it up. It may also be that the occasional slight acceleration instead of antagonism noticed by Bearn and Cramer (85) in ferment solutions may also occur in Opsonin solutions.

II. THE RELATIONSHIP BETWEEN OPSONIN AND IMMUNITY.

The reason why the opsonins have failed to remain in the high position in which Wright placed them as the real cause of immunity, is especially because they have been shown to exist in a generous measure beside susceptibility. Sleeswijk, for example has found a higher degree of phagocytosis of anthrax bacteria, with rabbit serum than with dog serum, the rabbit being highly susceptible and the dog immune to that disease.

In order to explain this fact, it is necessary to go deeper into the nature of opsonin, to find out particularly:-

A. in what way the opsonin of normal serum, of immune serum and of infected serum in which real immunity has not been established, differ from one another.

B. Whether there are any conditions which may hinder phagocytosis, either by acting on the bacteria, the opsonin, or the leucocytes.

A. Leishman (8) and Neufeld and Rimpau (31) have proved that the opsonin of immune serum differs from that of Normal Serum in that it is very much more stable. Heated Immune Serum gives a higher count than unheated normal serum, it may be even three

times as great. When Immune Serum is heated to 56°, it may be much reduced in amount, but is still considerable. When Normal Serum is heated at 56° almost all the Opsonin disappears, although as Dean (16), Klien (62), Fornet (39), and others have pointed out, a small residue remains. Levaditi (75-76), Dean (17-18), Böhme (59), and others have reactivated heated immune serum with fresh complement. Of those authors who have reactivated heated normal serum, the most marked results have been given by Cowie and Chapin (55). Unfortunately Cowie & Chapin used staphylococci and it is not easy to guarantee a serum as normal to staphylococci, so common are these infections. I have only twice tried to reactivate immune serum and succeeded both times in obtaining a reactivation.

EXP.	NORMAL GUINEAPIG SERUM +0.85% NaCl	HEATED IMMUNE GUINEAPIG SERUM +0.85% NaCl.	HEATED IMMUNE + FRESH NORMAL
7	0.76	1.28	3.14
	0.78	1.28	3.46

Pyocyaneus Bacteria.

This experience is much too small to build a theory on. The immune guinea-pig serum with 0.85% NaCl gave such an extreme degree of phagocytosis that it was impossible to count. Still it may be that immune serum differs essentially from normal in this respect. It will be interesting to discover whether the/

the serum of persons who have been infected by a long standing disease, such as tuberculosis where true immunity has had time to develop, but has not developed, conforms to this theory of immune serum or to the normal type which we have established in the earlier part of this work.

According to Wright (12) heated tuberculous serum contains a residue of Opsonin, while normal has none, but others do not agree with him in this.

I wish to compare the normal with tuberculous serum in this respect.

EXP.	NORMAL GUINEAPIG SERUM HEATED 56°	N.G.S. HEATED 60°	TUBERCULOUS GUINEA-PIG SERUM HEATED 56°	TUB.G.S. HEATED 60°	0.85% NaCl
8	1.52	0.64	1.34	0.78	0.62
9	1.18	0.9	2.0	0.78	
	NORMAL HUMAN SERUM HEATED 56°	60°	TUB.HUMAN SERUM HTD. 56°	60°	
19			0.56		0.26
20	0.23	0.33	0.56	0.2	0.2
"			0.4	0.2	0.2
21	0.4		0.4		0.2
"	0.5		0.3		0.2
22	0.23		0.2		
23	0.1		0.13		0.1
24	0.35		1.1		0.1
"			0.15		0.1
54	0.2		0.63		0.1
57	0.4		0.3		0.03
58	0.06		0.16		0.06
60	0.3		0.3		0.1
"			0.1		0.1
62	0.25	0.1	0.3	0 16	0.06
63	0.2	0.1	1.3	0.8	0.1
"			0.23	0.5	0.1
55	0.16		0.13		0.03
	0.1		0.1		
			0.03		
			0.03		
			0.1		
			0.13		
			0.36		

EXP.	NORMAL HUMAN SERUM HEATED 56°	60°	TUBERCULOSE HUMAN SERUM HEATED 56°	60°	0.85% NaCl.
64	0.2		0.7		0.06
"			0.3		0.06
65	0.23		0.16		0.1
"			0.23		0.1
66	0.06		0.1		0.03
67	0.06		0.36		0.03
"	0.1		0.33		0.03
69	0.2		0.3		0.06
"	0.06		0.1		0.06
70	0.03		0.2		0.0
"	0.03		0.17		0.0
71	0.1		0.3		0.03
72	0.1	0.03	0.14	0.06	0.0
"	0.12				
73	0.0		0.2		
	0.18		0.2		
74	0.08		0.1		0.0

In these tables, Tuberculose Serum which was always taken from long standing cases, is slightly more thermostabile than the Normal Serum, but not invariably so, and it is very little thermostabile in comparison with/

with serum which is really immune.

I have next tried to find whether the tubercular serum can be reactivated. One would expect, says Caulwell (56) to find the Amboceptor increased in such a case, and therefore reactivation may be more easily obtained. Caulwell was unable to reactivate normal serum, but obtained occasional results in the direction of reactivation, a minute increase such as 0.1 above the sum of the counts of diluted unheated serum and heated tuberculous serum. Occasionally, however, to negative this, there was an actual decrease.

EXP.	NORMAL GUINEA-PIG SERUM + NaCl.	TUBERCULOUS GUINEA-PIG SERUM HEATED 56°	TUBERCULOUS GUINEA-PIG SERUM HEATED + NaCl	TUBERCULOUS GUINEA-PIG SERUM HEATED 60°	FRESH NORMAL + TUBERCULOUS HEATED 56	FRESH NORMAL + TUBERCULOUS HEATED 60
8	2.8	1.52		0.64	1.92	1.6
9	2.4			0.78		1.16

NORMAL/

	NORMAL HUMAN SERUM + NaCl	TUBER. HUMAN SERUM 56° +NaCl	FRESH + NORMAL 56° + Nacl	TUB. HEATED. 60°
EXP. 19	5.7	0.56	2.0	3.2
21.	4.36	0.3	2.43	
"	4.36	0.4	2.53	
"	5.0	0.3	2.66	
"	5.0	0.43	2.53	
22	1.6	0.2	0.83	
23	2.65	0.1	1.2	
32	1.83		1.3	
54	2.5	0.63	1.45	
55	1.86	0.13	1.7	
57	1.56	0.3	0.85	
64	4.09	0.7	2.5	
		0.3	2.6	
75	1.3	0.8	0.93	

	NORMAL GUINEAPIG Dilution Serum diluted + NaCl.	TUB. GUIN. PG. SER. Heated. 56 +NaCl	60 +NaCl	FRESH & HEATED. 56°	60°
8	1/100	0.78	1.52	0.64	1.0	0.72
9	1/50	0.32	2.0		1.66	

EXP.	NORMAL G.PG. Serum 1/20 + NaCl.	TUBERCULOUS HUMAN 56° + NaCl	60° + NaCl	FRESH & HEATED. 56° +	60°
65	0.32	0.23	0.16	0.45	0.63
66	0.26	0.1		0.06	
	NORMAL RABT. Serum 1/12 + Nacl	+			
63	2.3	1.3	0.8	1.13	2.6
	2.3	0.5	0.23	2.23	1.6
	NORMAL HUMN. Serum diluted + NaCl.				
57	1/20 0.8	0.3		0.68	
58	" 0.54	0.16		0.41	
60	" 0.6	0.3		0.75	
"	" 0.6	0.1		0.43	
67	" 0.2	0.36		0.22	
"	" 0.2	0.3		0.4	
69	" 0.08	0.3		0.25	
"	" 0.08	0.1		0.25	
70	" 0.2	0.2		0.25	
"	" 0.2	0.17		0.26	
71	" 0.36	0.3	0.3	0.33	0.3
72	" 0.3	0.14	0.06	0.27	0.05

There/

There are only two cases here where the result of mixing fresh and heated serum give a result higher than the sum of their counts alone, the one is falsified by the fact that the same serum heated 56 instead of 60 gives no reactivation, the other shows an increase of 0.07 which is within the margin of error, in fact both are within the margin of error. The other cases show not only no reactivation, but in most cases an antagonism, just like that seen by normal heated serum. Especially where the serum is undiluted the antagonism exerted by Heated Tuberculous Serum is greater than that by Heated Normal Serum, and is more regularly met with. The opsonic power of guineapig serum is greater than that of human serum, it appears also more thermostabile, but the antagonism exerted by it is more regular, and more noticeable in the case of diluted serum,

The heated serum is not antagonistic to other heated serum but only to unheated .

	NORM.GPG.SERUM heated 60° + NaCl		Tub.GPG.SERUM heated 60° + NaCl.		
8	0.64	+	0.78	=	0.76
9	0.9+0.9			=	2.32
"			0.78 + 0.78	=	2.0
	heated 56° + NaCl		heated 56 + NaCl	-	
9	1.18+1.18			=	3.86
"			2.0 + 2.0	=	3.28

EXPT.	NORMAL HUMAN SERUM heated 60 + NaCl.		N.H.S. heated 56 + NaCl.	
13	0.23	+	0.23	= 0.33
15	0.1+0.1			= 0.2
"			0.7+0.7	= 0.7
16			0.53+0.53	= 0.56
19	0.1+0.1			= 0.2
"			0.56+0.56	= 0.6
9	1.42+1.42			= 2.02
9			1.54+1.54	= 1.82

Between Guineapig Serum 60° + 56° there appears an antagonism, but this is no doubt due to the fact that when Guineapig Serum is heated to 56° not so much opsonin is destroyed as in Human Serum at this temperature.

GUINEAPIG SERUM 60°		GUINEAPIG SERUM 56°	
0.64 -	+	1.34	= 0.64
0.78	+	1.52	= 0.56
	+	1.34	= 0.7
0.78	+	1.18	= 1.0

Heterogeneous serums, as Bächer (44) & Hamburger and Hekma (46) have pointed out, may inhibit phagocytosis, whether/

whether heated or unheated this appears undoubtedly to exist, whether through an influence on the leucocyte or not I do not know. The Guineapig Serum at 60° and the Human Serum at 56° lose almost all their opsonic power, and at the same time a liability to be antagonised. At the same time they gain a power to antagonise. We have T.B.thermostabile like N.S. also antagonistic (more so) against Fresh not heated in both.

I wished next to consider whether the variation of this antagonism depends on a varying susceptibility of the Unheated Serum or a varying strength of antagonism on the part of the Heated Serum.

EXP.	NOR.GP. SERUM + NaCl.	TUB.GP. SERUM + NaCl.	N.G.S. 60° + NaCl	TUB.G.S. 60° + NaCl	N.HUM. SERUM 60° + NaCl	FRESH & HEATED.
8.	2.8		+	0.64		= 1.6
	2.8		+ 0.78			= 0.7
		2.06	+	0.64		= 1.2
		2.06	+ 0.78			= 1.2
9.	2.7		+ 0.9			= 2.2
	2.7		+	0.78		= 1.16
	2.7		+		1.42	= 1.5
		5.83	+		1.42	= 1.5

EXP.	NORMAL HUMAN SER. + NaCl	TUB.HUM. SERUM + NaCl		N.H.S. heated 56° + NaCl	TUB.H.S. heated 56° + NaCl.		FRESH & HEATED.
19		5.6	+		0.56	=	1.0
	5.7		+		0.56	=	2.0
20	3.5		+	0.23		=	2.5
		3.56	+	0.23		=	2.7
21	5.0		+	0.4		=	4.23
	5.0		+	0.5		=	4.0
	5.0		+		0.4	=	2.66
	5.0		+		0.3	=	2.53
		3.76	+	0.4		=	3.2
		3.76	+	0.5		=	3.06
		3.76	+		0.4	=	2.23
		3.76	+		0.3	=	2.23
	4.36		+	0.4		=	3.63
	4.36		+	0.5		=	3.73
	4.36		+		0.4	=	3.53
	4.36		+		0.3	=	2.43
		5.16	+	0.4		=	4.33
		5.16	+	0.5		=	4.44
		5.16	+		0.4	=	2.33
		5.16	+		0.3	=	2.5
22.	1.6		+	0.23		=	1.33
	1.6		+		0.2	=	0.83
23		1.7	+	0.13		=	1.0
		1.7	+		0.1	=	0.56
	2.65		+	0.13		=	1.2
	2.65		+		0.1	=	1.0

EXP.	NORMAL HUM. SER. + NaCl	TUB.HUM. SERUM + NaCl		N.H.S. heated 56° + NaCl.	TUB.H.S. heated 56° + NaCl.		FRESH & heated.
32	1.83		+			=	1.26
	1.83		+			=	1.3
		1.2	+			=	0.7
54	2.5		+	0.2		=	2.08
	2.5		+		0.63	=	1.45
		2.42	+	0.2		=	1.8
		2.42	+		0.63	=	1.7
55	1.86		+	0.16		=	1.5
	1.86		+		0.13	=	1.7
		1.43	+		0.13	=	2.0
64	4.09		+	0.2		=	2.75
	4.09		+		0.7	=	2.5
	4.09		+		0.3	=	2.6
65	1.54		+	0.23		=	1.0
	1.54		+		0.23	=	1.26
66	1.2		+	0.06		=	0.5
	1.2		+		0.1	=	0.82
67	1.27		+	0.06		=	0.66
	1.27		+	0.1		=	1.02
	1.27		+		0.36	=	0.38
	1.27		+		0.33	=	0.86
70	0.7		+	0.03		=	0.72
	0.7		+	0.03		=	0.66
	0.7		+		0.2	=	0.55
73	1.23		+	0.03		=	1.26
	1.23		+	0.0		=	0.84
	1.23		+		0.2	=	0.2
	1.23		+		0.2	=	0.8
74	1.5		+	0.03		=	1.2
	1.5		+	0.0		=	0.86
	1.5		+		0.06	=	0.5
	1.5		+		0.06	=	0.53
75	1.3		+	0.08		=	0.93
	1.3				0.1	=	0.83

In almost all cases the Tuberculous Heated Serum is more antagonistic than the Heated Normal Serum.

The/

The instances in which the antagonism was greatest, were from very advanced cases, while it was less in certain less advanced cases, although all specimens came from long standing cases. But not only is the heated Tuberculose serum more antagonistic, but Unheated Fresh Tuberculous serum appears to be more susceptible to antagonism than is Normal serum.

B. CONDITIONS UNFAVOURABLY AFFECTING PHAGOCYTOSIS.

One such condition has already been proved, namely the presence of heated serum. Heated serum is antagonistic to phagocytosis if Fresh Serum be present, it appears, therefore, anti-opsonic rather than anti-phagocytic. It is necessary to go deeper into the nature of this antagonism and to decide whether it act on the bacteria, the opsonin or the leucocytes. All bodies which are receptors of the second order such as toxins or agglutinins, are inhibited by heated solutions of the same. Heat is supposed to injure the nucleus in such a way that only the binding part of it is left entire. The active part is damaged and the nucleus, therefore, inert, but the binding radicle is able to unite itself to bacterial receptors, or in other words, "to stop them up", so that undamaged nuclei will not be able to reach them. Hektoen and Ruediger (19)

(19) believe that opsonin is like agglutinin in this respect, that when opsonin is heated, it loses its power of opsonising, but not of fixing itself to the bacteria. There are three ways of proving whether an opsonised formation is the cause of the antagonism. (1) By treating bacterial cultures with Heated Serum for ½ an hour and then with Fresh Serum, and comparing this result with the effect of adding Heated and Fresh Serum to the Bacteria at the same time. (2) By treating serum with Heated Serum and allowing them to stand in contact for half an hour or longer, before bacteria and leucocytes are added, and comparing the result with the effect of adding Heated Serum and Fresh Serum to the bacteria and leucocytes at once.

If the table[5] given on pages 69–70 are examined, it will be seen that bacteria treated with heated serum and then washed are afterwards less easily phagocytised by fresh serum than untreated bacteria. On the other hand, all cultures which have been treated with serum and then washed and then treated with fresh serum, do not seem to be so susceptible as untreated Bacteria. In view of the fact that Bacteria are much more susceptible to opsonin, if first washed with salt solution, this is a curious phenomenon and will be later discussed under another heading. Heated serum, however,/

however, does not seem to inhibit bacteria more than unheated serum. Nor is the inhibition greater than that resulting from the addition of Heated and Unheated Serum together to bacteria, it cannot at least be said that it is greater as a rule.

EXP.	N.H.S. + untreated Bacteria.	N.H.S. + Bacteria treated with heated Serum.	N.H.S. + Heated Ser.
36	2.8	1.85	1.5
44	10.0	5.86	7.0
82	0.6	0.5	0.4
83	0.83	0.85	0.8
86	0.5	0.8	0.06
87	1.1	1.2	1.23
"	0.5	0.4	0.4
91	12.4	3.5	4.8

FRESH SERUM AND HEATED SERUM ALLOWED TO STAND IN CONTACT FOR SOME TIME BEFORE BACTERIA ARE ADDED.

EXP.	UNHEATED SERUM + NaCl.	HEATED 56° + NaCl	UNHEATED + HEATED	MIXT. of UNHTD. + HTD. aft. stand. in contact	Length of contact.	TEMPERATURE.
25	3.86 + 1/1	---	= 2.7	1.03	19 hrs.	10°-15°
	" + 1/10	---	= 3.03	3.3	" "	"
	" + 1/100	---	= 3.93	2.66	"	"
	" + 1/500	---	= ----	3.83	"	"
26	2.66 + 1/1	---	= 1.43	0.36	¼ hr.	"
27	1.53 + 1/1	---	= 1.3	0.8	"	"
	" + 1/10	---	= ----	1.4	"	"
	" + 1/100	---	= ----	1.46	"	"
106	3.23 + 1/1	0.52	= 1.07	2.3	30 hrs.	37°
	[illegible]			1.93	30 "	10°-15°
				0.9	¼ hr.	"
107	8.0 + 1/1	6.0	6.33	6.83	30 hrs.	37°
				6.5	30 "	10°-15°

These few examples appear to indicate that by bringing Fresh and heated Serum in contact before the bacteria are added, antagonism is intensified. If, however, the contact is as long as 30 hours the Opsonin appears somewhat revived, especially if the mixture/

mixture has been incubated at 37°.

No proof of opsinoid formation can, so far be deduced.

(2) Welsh & Chapman (96 & 97) find that if precipitin serum be heated to 72° for ½ an hour, it becomes inactive, but not inhibitory. If heated to 75° for half an hour it becomes both inactive and inhibitory. In such a case a Precipitoid explanation is impossible. I have attempted to discover a temperature at which opsinoid is inactive, but not inhibitory, but without success.

EXPT/

EX.	NORMAL HUMAN SERUM + NaCl.	N.H.S.1/20 diluted + NaCl.	N.H.S. heated 51° + NaCl.	N.H.S. 53° 54° + NaCl	N.H.S. 56° + NaCl	FRESH + HEATED.
46	4.0		2.3			-
	4.0			1.06		3.0
	4.0				1.0	3.5
57	1.56		0.95			2.0
	1.56			0.3		0.96
	1.56				0.4	1.5
	1.56		0.9			1.9
	1.56			0.5		1.44
	1.56				0.3	0.85
58	2.5	;(0.54)		0.3		0.26
	2.5	;(0.54)			0.06	0.68
	2.5	; 0.54		1.05		0.62
	2.5	; 0.54			0.16	0.68
60	2.13	; 0.6		0.33		0.5
	2.13	; 0.6			0.3	0.8
	2.0	; 0.6		0.53		1.0
	2.0	; 0.6			0.3	0.75
	1.8	; 0.6		0.23		0.6
	1.8	; 0.6			0.1	0.43
62	1.5	; 0.7		0.2		0.66
	1.5	; 0.7			0.25	0.46
	1.2	; 0.7		0.2		0.46
	1.2	; 0.7			0.3	0.4

The diluted serum was not necessarily from the same source as the undiluted, it was only introduced for the antagonism. Although the experiment is not against an opsonoid formation, neither is it for it.

(3) Although I do not approve in the least of Ehrlich's theory of Bacterial Receptors as applied to Opsonin-Absorption for reasons which will later be discussed, yet arguing from the basis of this theory for once, were the diluted heated serum added to diluted unheated, there should be room and to spare among the bacterial receptors for both opsonin and opsinoid, so that no antagonism ought to take place such as one gets if diluted or undiluted unheated serum is added to undiluted heated. According to Ehrlich's theory if antagonism be still evident between diluted unheated and diluted heated, the opsinoid explanation of the antagonism becomes impossible.

EXP.	DILUTION	N.G.S. + NaCl		N.G.S. heated 60° + NaCl		DILUTED HEATED + UNDILUTED UNHTD.
1.	1/10	2.42	+	1/10	1.25	= 1.2
	1/50	1.25	+		1.25	= 1.2
	1/100	0.6	+		1.25	= 0.7
3	1/10	2.2	+	1/10	1.75	= 1.44
	1/50	1.9	+		1.75	= 1.4
	1/100	1.3	+		1,75	= 0.8
4	1/10	0.96	+	1/10	0.8	= 1.06
	1/50	0.8	+		0.8	= 1.06

EXP.	DILUTION	N.G.S. - NaCl.	DILUTION	N.G.S. heated 60 + NaCl		DILUTED HEATED + UNDIL. UNHEATED.
4.	1/100	0.7 -	1/10	0.8	=	0.92
4	1/100	0.7	1/50	0.7	=	0.8
6	1/10	3.24	1/10	0.9	=	2.22
"	1/50	2.6	"	0.9	=	0.9
"	1/50	2.6	1/50	0.96	=	1.04
"	1/100	1.6	"	0.96	=	0.9
"	1/10	3.24	1/100	0.84	=	2.5
"	1/50	2.6	"	0.84	=	1.04
"	1/100	1.6	"	0.84	=	0.4
"	1/10	3.24	1/500	0.9	=	2.68
"	1/50	2.6	"	0.9	=	1.26
5	1/10	2.64	1/10	2.3	=	1.3
"	1/50	1.96	"	2.3.	=	1.36
"	1/100	1.5	"	2.3	=	1.08
"	1/100	1.5	1/50	1.1	=	1.4
		N.H.S. + NaCl		N.H.S. heated 60 + NaCl		
14	1/10	0.4	1/10	0.03	=	0.43
	"	0.4	1/50	0.0	=	0.43
15	1./10	2.0	1/10	0.25	=	1.4
16	1/10	2.1	"	0.2	=	2.10
17	1/10	2.5	"	0.4	=	1.73
19	1/10	4.1	"	0.3	=	0.7 TUB. SER.
77	1/10	1.8	"	0.3	=	0.7

The antagonism between diluted heated and diluted unheated serum is quite striking, especially in the examples of Guineapig or Tuberculous human serum. I have, therefore, managed to obtain no satisfactory proof that the antagonism is due to an opsinoid formation - and one satisfactory against it. If the antagonism act against the opsonin, it might be thought that heated serum contains, perhaps a precipitate of opsonin, which when added to fresh serum induces a farther precipitate of opsonin in that. This cannot, however, be the case, because the antagonism has been shown to diminish the more the serum is diluted, also if the serum be left in contact with diluted heated serum over night, as has been already cited, this diminishing antagonism is still evident.

C). THE ACTION OF HEATED SERUM ON THE LEUCOCYTES.

It has already been pointed out that heated serum alone increases rather than diminishes phagocytosis. This does not favour the idea that heated serum is detrimental to the leucocytes.

Leucocytes were treated with heated serum for half an hour and then washed.

EXP./

EXP.	NORMAL HUMAN SERUM + untreated Leucocytes	NORMAL HUMAN SERUM + treated Leucocytes.
29	3.5	1.93
	5.5	2.1
44	10.0	8.3

The comparison of different emulsions of blood leucocytes, even from the same blood, and even where all precautions are taken to wash and centrifuge and separate each equally, is not very accurate. It can well be, however, that the heated serum is able partly to be absorbed on the surface of the leucocytes and again to be given off in presence of habile opsonins.

OTHER CONDITIONS HINDERING PHAGOCYTOSIS.

The hindrance to phagocytosis produced by heated serum, while interesting, is yet artificial. It is necessary also to consider anti-phagocytic conditions which may arise in the body fluids themselves. In an infected organism bacteria are living and secreting toxic substances, and certain of these toxic substances have been shown to hinder phagocytosis by repelling the leucocytes. In 1902 Bail, & Petterson (24)/

(24) who had done much work on the Amboceptor as it behaves in vivo rather than in vitro, came to the conclusion that there was no relationship between immunity and the amount of Amboceptor present. In 1904 he formulated the theory that the Bacteria produce an especial secretion which is the expression of its virulence. He called this Aggressin and believed immunity to be due to a theoretical Anti-aggressin. Working with tuberculous guineapigs in 1905 (27) he discovered that instead of a tolerance being produced by the inoculation or auto-inoculation of Aggressin, in this case an actual hyper-sensitiveness to Aggressin was produced. He notices that if 1/100 mg Tubercle culture were inoculated into the peritoneal cavity of a normal guineapig, the leucocytes crowded to the place and took up the bacteria, and a chronic infection ensued. If it were an already tuberculous animal the leucocytes did not crowd to the spot, and an acute infection followed with rapid death. If, however, the bacteria were washed, the leucocytes were not repelled, and phagocytosis took place. In 1906 Levy and Fornet (49) found that the aggressins could filter. I have used Tubercle and Pyocyaneus Culture filtrates in the following experiments, also old Koch's Tuberculin 1/10,000.

I./

I. The EFFECT of such a FILTRATE on the PHAGOCYTOSIS of NaCl. in presence of

EXP.	2 parts of NaCl.	1 part of NaCl + 1 part of Filtrate.	
7	0.88	0.24	Tub.
8	0.62	0.42	"
"	0.62	0.38	Tuberculin
7	0.88	0.98	"
7	0.3	0.56	Pyocyan.
82	0.1	0.06	"
83	0.03	0.03	"

II. The EFFECT on the PHAGOCYTOSIS of OPSONIN.

	FRESH SERUM + NaCl.		TUB.FILTRATE + NaCl.		NORMAL SERUM + FILTRATE.
7.	3.48	+	0.24	=	1.02
8	2.06	+	0.42	=	0.52
"	2.8	+	0.42	=	0.46
29	5.5	+	–	=	2.1
	4.25	+	–	=	2.85
30	2.8	+	–	=	1.53
32	1.83	+	–	=	1.5

			TUBERCULIN + NaCl.		FRESH SERUM + TUBERCULIN
8	2.06	+	0.38	=	0.9
"	2.8	+	0.38	=	0.74
			PYOCYANEUS FIL. + NaCl		
7	0.76	+	0.56	=	0.5
82	3.6	+	0.06	÷	0.4
"	¼ 0.6	+	0.06	==	0.0
83	0.83	+	0.03	=	0.3
84	1.6	+		=	0.35

It is clear from these few examples that phagocytosis is powerfully affected by bacterial products, it appears likely not only from the work of Bail (27, 28. 29) etc. but also from the fact that the spontaneous phagocytosis of NaCl is reduced that the action is mainly against the leucocytes. I have noticed also that unwashed cultures especially of pyocyaneus are very much less easily phagocytised than washed cultures, by normal serum. Many instances may be found earlier in this paper to show that the count with opsonin may be very much higher than of Nacl where washed bacteria have been used. In one case with unwashed bacteria, the normal serum gave a count very little higher than NaCl, namely 0.76 to 0.3 while Immune Serum (to pyocyaneus) gave such a great degree of phagocytosis that it could not possibly be counted. It is interesting that Neufeld believes his Bacteriotropin, the phagocytosis furthering constituent of Immune Serum, to be identical with the theoretical Anti-aggressin of Bail.

It is a curious fact that although the bacteria are better prepared for phagocytosis if they are washed with 0.85% NaCl, yet are distinctly inhibited if they are instead treated with serum heated or unheated whether at 37° or 0°. It is unlikely that"receptors" are washed off.

It/

It appears possible that if the normal opsonin be left in contact with the bacteria too long before phagocytosis takes place, a change occurs in it. Where in the body fluids the aggressins are repelling the leucocytes, we repeat this state of affairs artificially by treating bacteria with serum in the absence of leucocytes. It is true that in the artificial case we wash the bacteria and in the body fluids this does not take place, but the fact that the bacteria are not fully opsonised at the end of the treatment does not necessarily mean that opsonin has been washed away. It is possible that something else has happened. I must protest that it is impossible to regard the theory of bacterial receptors as any explanation of the absorption phenomena of opsonin. We can have a serum with a very thick emulsion of bacteria giving a very large count, say 60 bacteria per leucocyte, and the same serum with a thin emulsion giving a count of 1 bacterium per leucocyte, this might be explained by Ehrlich as sufficient receptors in the first case and insufficient in the second. But why then does Serum B. differ from Serum A. not only with the thick emulsion, but also with the thin? If we were dealing with affinities between bacterial receptors and opsonin, there would be no difference whatever between serums provided the/

the bacterial receptors were insufficient. Everything would be levelled, and this is not the case, as every worker must have experienced. There is a very much simpler explanation than that of bacterial receptors. Substances are taken up from solutions or suspensions on to solid surfaces in obedience to a physical law which is expressed by the formula $C_1=KC_2^n$ where C_1 is the concentration of the solute in the liquid, C_2 the concentration in the solid, n & K are constants depending on the chemical nature of the substances. If n be more than 1, and a total concentration C_A be greater than C_B, then the difference between C_{1A} and C_{2A} will be greater than between C_{1B} and C_{2B}. This is the only explanation of the fact that the difference between Serum A. & B. is always demonstrable. Whether the bacteria are many or few opsonin is absorbed on all and the more concentrated the opsonin, the more will be absorbed in geometrical ratio. Considering that the relation between free and absorbed opsonin remains the same if the concentration of total opsonin is the same, whatever the number of bacteria, and considering that if there be many bacteria the absolute amount of free opsonin is lowered and of opsonin in each bacterium, it appears at first puzzling why so many more bacteria are phagocytised from thick emulsions/

emulsions than from thin. Other conditions assisting phagocytosis are, however, increased in thick emulsion, i.e. tendency to spontaneous agglutination, and proximity of bacteria to leucocytes. It is also probable that although absolutely more bacteria are taken up from thick emulsions relatively fewer are taken up.

The surface of the bacterium is a presumably porous one, it is in fact a membrane. It must be remembered that passage through a membrane is not a mechanical one depending on the size of molecules, as was once taught. The contrary has been proved by Nernst (99) Ramsay (100) Crum Brown (101) and others. The substance is taken up by the membrane in solution and forms a loose union with it. If a substance can unite with its colloidal walls it can dialyse through them, and vice versa. The simplest illustration is Ramsay's, at a particular temperature Hydrogen will unite with Platinum and only at that temperature will it dialyse through it. If a substance pass into solution, it is not always the same substance which comes out of it, therefore, if a substance pass through the pores of a colloidal membrane, it is not necessarily the same substance which is given off at the other side. Also if the substance be changed by this solution it will be given off in the changed condition on both sides

of/

of the membrane. If we cut the colloidal membrane into small pieces, the substance will still dialyse, and will change or not according to its nature.

We have seen that opsonin is absorbed into the pores of the bacterial membrane, it disappears at the same time out of the serum to a corresponding extent. If Opsonin be allowed to remain in this condition for some time before phagocytosis can take place, the bacteria are not well phagocytised when leucocytes are added. They are also less sensitive to fresh opsonin. Later also serum which has been treated becomes gradually also somewhat antagonistic. This fact has also been noticed by Ledingham (103) who, however, explained it by supposing that Bacterial receptors were thrown off, which deviated an Amboceptor. We have already discussed Bacterial Receptors.

SOME ILLUSTRATIONS of THIS FACT.

EXP.	NORMAL HUM.SER.	SER.treated wth Bact. at 0°	SER.treated with Bacteria at 37°	FRESH + TREATED.	
23	2.65	0.16		= 0.9)	
33	1.4	0.25		= 0.7)	
37	1.96		0.26	= 2.03)	
41	($\frac{1}{5}$)5.7	$\frac{1}{5}$ -		= 0.3)	
"	" 5.7		($\frac{1}{5}$) 0.1	= 0.1)	TUBER-
50	2.7	1.4		= 2.2)	CLE
50a	2.85	1.4 4		= 2.0)	
56	1.44	0.6		= 1,06)	
77	2.7	0.3		= 1.98)	
106	3.23	0.98		= 1.4)	
85	1.8	0.4		= 1.5	
87	1.1	0.6		= 1.23	PYOCYA-
"	($\frac{1}{4}$)0.5	$\frac{1}{4}$ 0.6		= 0.4	NEUS
107	8.4	4.25		= 5.0)	PARA- TYPH.

It is interesting in this connection that Wright (11) has described areas of "lowered bacteriotropic pressure" in the neighbourhood of tuberculous foci, where the opsonin has been exhausted. The injection of tuberculin in the tuberculous subject where an over-sensitiveness to Aggressin has been developed, is well known to cause a fall in the opsonic index, known as the negative phase.

We have also seen that if serum be dialysed through Gelatin, Collodion or Reed sacs or films, the opsonin disappears upon one side, and an antagonistic substance appears on the other. One's attention is attracted by the similarity of these phenomena. It is essential to consider where this antagonistic substance comes from, whether the body fluids contain naturally such an anti-immune substance, or whether it is produced by pathological conditions only. The serum after heating at 56° for $\frac{1}{2}$ an hour is still opsonic. It, therefore, contains in small amount an opsonin which is thermostable. It is, however, also anti-opsonic, not to other heated serum but to unheated. We cannot regard the small opsonic power of heated serum as explained by an excess of thermostable antagonisable opsonin over antiopsonin, because in that case, the effect of all the anti-opsonin would be neutralised. We are forced, therefore, to believe that the small opsonic count at 56° is due to an opsonin differing/

differing from Normal Labile opsonin not only in its greater stability to heat, but also in the fact that it is not antagonisable by this anti-opsonic substance. What then is the origin of this anti-opsonin, and how does it exist in Fresh Serum.

Is it (1) a thermostable substance freed only by certain conditions from its union with Normal Labile opsonin by which it is much more than neutralised. (2) An inactive pre-antiopsonin converted by certain conditions such as heat, into anti-opsonin at the same time as opsonin is destroyed. (3) The stable form into which Labile opsonin is itself converted by heat.

The count of unheated and heated serum together is still much greater in most cases than that of heated serum alone, and cannot, therefore be attributed to it.

The anti-opsonin is, in fact, less than the labile Opsonin. If the anti-opsonin were to break off from its combination with the labile Opsonin and dialyse through a membrane and spread itself over the salt solution outside the sac as well as inside, the Serum inside would increase, if anything, in opsonic power, taking for granted that if the labile Opsonin did not dialyse it would not be absorbed by the colloidal walls. Dialysed Serum does not, however, increase even when the sac is suspended in 20cc of

0·85%/

0·85% NaCl which is constantly renewed in order to remove the anti-opsonin.

If a pre-anti-opsonin were converted into an active modification by heat and by dialysis, this anti-opsonin being already proved less and not more than labile Opsonin in amount, then the Serum inside the sac would contain all the labile Opsonin and a part of the activated anti-opsonin and would not, as a whole, be antagonistic to fresh Serum, as heated Serum where the labile Opsonin is destroyed would be. The dialysed Serum does, however, in most cases, become antagonistic to fresh Serum (p.64). Before accepting the third theory it would be interesting to compare the antagonism of anti-opsonin with what is known of the antagonism of anti-ferments generally.

A ferment is a catalyst, hastening an action which would take place slowly without it. Opsonin has been proved by Löhlein (50) and others to hasten phagocytosis which takes place more or less in its absence. The end which the catalyst hastens is an equilibrium towards which two processes tend, the one being the reverse of the other. Either process the catalyst hastens. The direction in which it acts does not depend, so far as we know, upon any modification of the catalyst, it depends only upon which side of equilibrium the process stands. We should not expect that the position of the process remaining the same, a modification of Opsonin should determine the direction

of/

of this process. At one time negative catalysts were spoken of, that is to say catalysts able to act in a direction away from equilibrium, but the last example in chemistry has now been explained away. This was the case of oxidation from Sulphite to Sulphate which was inhibited in the presence of alcohol, etc. It has, however, been proved that the alcohol is no negative catalyst, but acts by forming complexes with the catalyser, a minute trace of copper ion which is thus prevented from acting (108). It is unlikely, whatever the source of the anti-opsonin that it act as a negative catalyst.

Opsonin has other properties in common with Ferments. Not only is it thermolabile in solution and thermostable when dried (61) but it displays certain absorption properties. These absorption properties appear to enable the Ferment to perform its action, on the one hand, and on the other, to lead to its inhibition. Ferment and Opsonin too may be carried down by certain precipitates, their activity being in this way temporarily suspended. They may also absorb anti-ferments. There are two kinds of anti-ferments, and there are two ways in which Ferments may be affected by them. The first is where the anti-ferment is itself a ferment which is able to decompose its Ferment-host, in this case the Ferment-host is destroyed. In the/

the second case the anti-ferment is not able to decompose the Ferment, but is able only to inhibit its action so long as it remains in its pores. Examples of the first are seen in the action of proteolytic enzyme of yeast-cells on Buchner's Zymase, and the action of Pepsin upon Trypsin and diastase (102). Examples of the second case have been largely worked at by Bredig (78) for colloidal Platinum in its action on H_2O_2. Bredig finds that a large number of inorganic substances, for example, HCN and H_2S are able to inhibit this catalytic action. The Platinum is, however, able to recover itself by oxidizing the anti-ferment. Dastre and Stassano (104) have shown that when Trypsin has absorbed anti-trypsin, it is able slowly to recover, in less than ten hours ferment action is again perceptible. Spiro (92) finds that when Rennet and Anti-rennet have stood together for a short time (not specified) recovery begins to take place. It will be seen by reference to page (90) after 30 hours standing together with Anti-opsonin, especially at 37°, a certain recovery of Opsonin takes place. This appears to indicate that Opsonin belongs to the second class of anti-ferments.

The order in which Ferment and Anti-ferment are added to the substrate is of importance. Schönbein (78), as early as 1867, showed that although the anti-ferment/

anti-ferment acts against the Ferment and not on the substrate, yet, by adding HCN first to H_2O_2 and later blood cells, the action of the anti-ferment was much intensified. This has been later proved by Bredig (78) for Platinum anti-ferments. It is extremely interesting that Zangger (117) has noticed that the order of addition is of great importance in the haemolytic effect of Saponine and Sodium taurocholate. Each is haemolytic alone, the effect is enhanced or annulled when both are added, depending on the order of addition. In the case of Opsonin, anti-opsonin is formed in the mixture of Opsonin and bacteria, but, as we have seen, the treatment of bacteria first with anti-opsonin does certainly cause inhibition.

The immune substances of the blood have two characteristics which are less perfectly those of ferments. The first is the reversibility of their combinations which has been well established for immune substances. Calmette (105), for example, has proved this for the toxin - anti-toxin bond, Ehrlich (5) for Complement, Madsen and Walbum (106) for ricin - anti-ricin, Morgenroth (107) for Amboceptor, and Eisenberg and Volk (73) for Agglutinin.

The second is the kind of work the Ferment and Immune Substance are called upon to perform. The Ferment appears either to decompose, or synthetise. Most/

Most.and probably all,immune substances dissolve, or precipitate. Solvents, such as Amboceptor and Complement, dissolving constituents of the membrane of bacteria or red cells, may act by decomposition although their action appears somewhat too rapid. Precipitants, however, such as agglutinin or precipitin, one is loath to put into the same class. According to the physical-chemical school of biologists death of the cell is due to precipitation of suspended colloids in the cell. Where two colloids meet and precipitate the general rule is that they are present in a particular concentration and have opposite electric charges. (114). When a dangerous colloid enters the blood serum, native colloids in a particular amount and with a particular electric charge will meet it, absorb it, and precipitate down with it. The foreign colloid suffering as all absorbed bodies from a peculiar liability to oxidation or decomposition, is then disposed of. This is a simple view of acquired Immunity, and one for which there is much to say. It is not necessary that a visible precipitate exist in the plasma or serum. Immunity is not associated with such precipitates. The serum is a heterogenous system, full of particles, which it is able to maintain in a fine state of suspension. Adsorption may take place upan their surfaces without cohesion or agglutination into a visible deposit I am forced to believe/

believe that the majority of immune substances act as precipitating agents. Complement and Amboceptor are exceptions. Into which class are we to put Opsonin? The great majority of authors believe that Opsonin is identical with Complement either alone or with Amboceptor. We have not the slightest proof that Opsonin is a solvent, we have here a fundamental difference between Opsonin and Complement. Opsonin, where Bacteriolysin is absent, produces no bacterium shadows. Opsonin with bacteria capable of being bacteriolysed, if Amboceptor and Complement have not had time to act, produces no bacterium shadows, while it is able to produce phagocytosis. If Opsonin were a solvent, like Complement, it would dissolve the bacterial wall and if it dissolved any constituent of the bacterial wall we would expect to see either empty bacterial shells, or if bands existed, partially emptied shells. As we see nothing of the kind, we have no reason to believe that Opsonin is a solvent. The theory of Keith (52) that haemo-opsonin acts by precipitating the red cells against the leucocytes has much in its favour. Neufeld (31) has noticed with Immune Opsonin (Bacteriotropin) that when cocci leucocytes and immune serum are mixed the leucocyte are rapidly filled with cocci and leucocytes and cocci agglutinate into large clumps, or balls. This does not occur with the leucocytes alone/

alone. In the case of Normal Serum, with a thick emulsion of bacteria, the bacteria appear to be massed over the leucocytes, especially clumps of leucocytes. There are often dense rings of bacteria round each leucocyte. Dean (16) has shown that agglutination of bacteria favours phagocytosis. Conditions favouring agglutination favour phagocytosis, such as a high concentration of bacteria, although, as we have already noticed, the amount of Opsonin per bacterium must be less where there are many bacteria. When it is remembered how extremely slow the movements of leucocytes are, that the leucocytes are not themselves stimulated, it becomes very difficult to believe that in 8 minutes the same leucocytes with the same bacteria can pick up 19 bacteria from one serum and only a few per leucocyte from another as Böhme has reported, if the only difference be a different strength of attraction or desirability on the part of the bacteria. It appears that Opsonin is intended to act between bacteria and leucocytes. In the absence of the leucocytes, the Opsonin lying in the pores of the bacteria and liable, as all absorbed bodies seem to be, to decomposition, suffers a change of such a kind that it is now anti-opsonic. The anti-opsonin will naturally be given off by the bacteria/

bacteria to the free fluid in order to keep equilibrium. It is absorbed by Opsonin and in the pores of Opsonin is finally decomposed.

It is interesting that Welsh and Chapman (97) have proved that heated precipitin produces an inhibitive body which is not a precipitoid and this is formed out of the precipitin itself. Normal Opsonin is perfectly capable of helping the phagocytosis of non-virulent bacteria. Hektoen and Ruediger (19) have clearly shown us that it has much less influence where the bacteria are virulent. The presence of aggressin in any quarter of the body will cause a repulsion of the leucocytes especially, for example, in the tuberculous organism where an oversensitiveness has been developed. The agglutinating properties of immune Opsonin only appear strong enough to overcome this. The measure of Normal Labile Opsonin can in itself give little clue to the protective power of a serum.

CONCLUSIONS/

CONCLUSIONS.

Phagocytosis is carried out in two stages. One is the stage of opsonising, the other of the ingestion of bacteria by leucocytes.

The function of Opsonin is to approximate bacteria and leucocytes to one another.

The process may be described as an agglutination of bacteria to leucocytes. Agglutinations of bacteria to bacteria, or of leucocytes to leucocytes only occur secondarily.

Not only is the function of normal Opsonin different from that of any other immune substances, but its constitution is different.

There are two Opsonins in Normal Serum, one is thermolabile, the other thermostable.

Labile Opsonin is capable of undergoing a change by heat and also by passage through a membrane which renders it not only inactive as Opsonin, but also in part inhibitory. The part of labile Opsonin which can become inhibitory varies in amount, and may be absent. The Inhibition is not due to an opsonoid, but to an anti-opsonic formation.

The Anti-opsonin acts like many anti-ferments by absorption into the pores of the Opsonin where it is gradually/

gradually decomposed.

There is no reason to suppose that thermostable normal Opsonin differs from thermostable Immune Opsonin. There is, however, every reason to suppose that Normal Labile Opsonin differs from thermostable Immune Opsonin.

Normal labile Opsonin has no possible connection with Complement except in so far as they have certain absorption properties in common. The two bodies can expecially be distinguished by their behaviour under the influence of Time dilution, acid, dialysis, treatment with bacteria at 0 .

Normal labile Opsonin is not identical with Amboceptor, nor has it an Amboceptor-Complement form.

The Serum of tuberculous individuals varies slightly from case to case, but follows much more closely the Normal than the truly Immune type. It is occasionally, but not invariably, more thermostable to a slight degree. The chief difference in the Opsonin distribution is that the potential anti-opsonin appears, as a rule, greater in tuberculous Serum.

From the undifferentiated measurement of Normal Opsonin little information can be got as to the protective powers of the Serum.

An individual who has much potential anti-opsonin has an in-born susceptibility to the particular disease.

When/

When the determining factor, the bacteria, are added, in a case where Bacteriolysis is impracticable, the issue depends upon the nature of their virulence, expressed by aggressins. If it is of such a kind as to call forth sufficient immune Opsonin to overcome the repulsion of the leucocytes the result will be favourable. If, instead, it produce an hypersensitiveness so that the leucocytes are more strongly repelled, and in their absence anti-opsonin be formed and it be much, the prognosis is less favourable.

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OPSONIC

EXPERIMENTS

For Reference.

Record of Opsonic Experiments.

Method.

I have used the quantitative method of Wright throughout these experiments with the following departures, or special preventive measures.

Blood for Serum has been collected in long straight capillary tubes instead of Wright's bent tubes, as I believe in this way less blood is required, and there is less danger of scorching the blood when sealing the ends.

Leucocytes. In experiments 1 to 9 guineapig leucocytes from peritoneal exudate were used, as in these experiments serum was principally used. They have been obtained in two ways, either by one injection of 5cc of bouillon containing aleuron, or after two injections of simple bouillon alone, one 18 hours, one 4 hours before extraction. By the second method I have been successful in obtaining as much as 50 cc exudate.

In all other experiments human leucocytes were used prepared according to Wright.

Bacteria Typhoid, paratyphoid and Pyocyaneus Bacteria were taken, from a 24 hours old agar Culture. Allen & Hanbury's moist Tubercle culture was used to some extent, also a home grown bouillon culture of tubercle. All Tubercle bacteria were used dead. Tubercle used in all experiments unless otherwise mentioned in footnote.

No standard, such as Cowie & Chapin & Meyer recommend, has been used in making up the bacterial emulsion. Where two emulsions were to be compared, they were standardised against each other. Dilute emulsions were always made, except in the case of some dilution experiments.

Where Serum has been heated, or has been treated with bacteria at any temperature, the treatment has lasted $\frac{1}{2}$ an hour, except in one or two specified instances. Where Serum has been treated with Bacteria at 0^{o}, special care has been taken in every instance except one (No.33) to cool both serum and bacteria at 0^{o} for one minute before mixing, and to centrifuge the mixture after $\frac{1}{2}$ hour's contact, in ice and K SO mixture, and to separate at 0^{o}. Wherever bacteria have been washed NaCl has been used, as I have noticed that the greater the concentration of NaCl at room temperature the less easily will bacteria precipitate either by time or by centrifuging.

Where Serum has been dialysed, Collodion and reed sacs have been used. Control serum has been kept under equal conditions as regards temperature and evaporation, both being covered by indiarubber caps.

0.85% NaCl has been used except in the case of the Pyocyaneus experiments where 1·2% NaCl was employed. In every instance four sections of fluid have been taken, one of leucocytes, one of bacteria, one of serum and one of NaCl, of other serum or fluid. In NaCl controls 2 sections of NaCl were taken. I have avoided in every instance taking any section from the surface either of the bacterial emulsion or of any other fluid.

I have unfortunately kept no record of the order in which the sections were taken. The order has I believe varied very much, as I did not attach at the time any significance to this.

Incubation of the capillary tubes lasted $\frac{1}{4}$ hour except in one or two specified instances.

I have fixed my films in the vapour of 2% osmic acid, or that of a mixture of strong formalin and acetic acid for 1 minute.

Staining With bacteria other than Tubercle, I have simply poured on Giemsa stain and left it for a quarter of an hour when a perfect contrast stain is secured as by that time the nuclei have become reddish as well as the protoplasm so that the bacteria are well-defined.

For Tubercle bacteria Ziehl-Neelsen's stain is perfectly useless because only the nuclei and the bacteria are stained and the limits of the cell-protoplasm can generally not be made out. I have therefore stained first with Ehrlich's acid Haematoxylin (Grublers) for $1\frac{1}{2}$ to 24 hours. After washing, boiling Carbolfuchsin is poured on the slides and left for 10 minutes. The slides are washed and then decolourised first in 2% Hydrochloride which does not affect the Haematoxylin, and then in Alcohol or Methylated Spirits. After washing, 1% watery Methylene blue is applied for $\frac{1}{2}$ - 1 minute. This stain gives beautiful results.

In the following experiment-schemes certain expressions have been used for the sake of brevity.
Index means, only, bacteria per leucocyte.
NHS is Normal Human Serum, NGS, normal guineapig serum, and NRS, normal rabbit serum.

EXPERIMENT, No I. 11-6-'07

No.	I Injected Guineapig Serum	II Guineapig Serum Heated 60°	III Guineapig Serum not heated 1/10	IV Heated 1/10	V Not heated 1/50	VI Not heated 1/100	Results: Percentage of Noughts	Results: Bacteria p. leucocyte	Remarks
1	*						17%	2·7	Index=number of
2		*					78	0·4	bacteria per leuco-
3			*				20	2·42	cyte.
4				*			47	1·25	Injected guineapig=
5					*		75	0·6	the animal injected
6						*	75	1·1	with Aleuron for
7		*	*				52	1·4	leucocyte exudate,
8		*			*		75	1·2	otherwise normal.
9		*				*	55	1·1	Incubated ½ hour at
10			*	*			55	1·2	37°.
11				*	*		55	1·2	All dilutions made
12				*		*	57	0·7	with 0.85% NaCl.

EXPERIMENT No II. 12-6-'07

No	I Injected Guineapig Serum	II I. Guineapig Serum 1/10	III Guineapig injected 1/50	IV I. Guineapig 1/100	V Normal Guineapig	VI Human Serum	Results: Percentage of Noughts	Results: Bacteria per leucocyte	Remarks
1	*						26%	2.2	"Injected"=injected
2		*					42	1.65	with Aleuron in 5 cc
3			*				51	1.35	bouillon for leuco-
4				*			62	1.0	cyte exudate, other-
5					*		26	2.0	wise normal.
6						*	75	0.5	Guineapig leucocytes incubated ¼ hour

EXPERIMENT No III. 12-6-'07.

No	I Injected Guineapig Serum	II I. Guineapig heated 60°	III Not heated 1/10	IV Heated 1/10	V Not heated 1/50	VI Not heated 1/100	Results: Percentage of Noughts	Results: Bacteria per Leucocyte	Remarks
1	*						14%	3.76	Guineapig leuco-
2		*					70	0.54	cytes incubated
3			*				30	2.2	¼ hour.
4				*			*bad specimen* 46	1.75 *bad specimen*	
5					*		43	1.9	
6						*	56	1.3	
7		*	*				48	1.6	
8		*			*		58	1.0	
9		*				*	36	1.4	
10			*	*			52	1.44	
11				*	*		38	1.4	
12				*		*	60	0.8	

EXPERIMENT No IV. 20-6-'07

No	I Injected Guineapig Serum	II I. G. S. heated 60°	III Not heated 1/10	IV Heated 1/10	V Not heated 1/50	VI Heated 1/50	VII Not heated 1/100	VIII Heated 1/100	IX NaCl	X	Result: Percentage of Noughts	Result: Bacteria per leucocyte.
1	*								*		*bad specimen*	
2		*							*		70%	0.5
3			*						*		52	.96
4				*					*		62	.8
5					*				*		36	.8
6						*			*		66	.7
7							*		*		*bad specimen* 72	.36
8								*	*		68	.62
9		*	*								56	.57
10		*			*						66	.62
11		*					*				68	.54
12				*	*						50	1.06
13				*			*				60	.92
14						*	*				58	.8
15									**		46	.55
16									*	*	50	.8

Injected Guineapig Serum, from animal injected with bouillon for exudate, otherwise normal. Serum heated to 60° for half hour.

EXPERIMENT No V. 28-6-'07

No	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	Results	
	Injected G. Serum	Injected heated 60°	Not heated 1/10	heated 1/10	Not heated 1/50	Heated 1/50	Not heated 1/100	Heated 1/100	Injected heated 58°	Normal heated 60°	Normal not heated	Normal heated 58°	NaCl	Percentage of Noughts	Bacteria per Leucocyte.
1	*												*	24%	3.4
2		*											*	60	.96
3			*										*	36	2.64
4				*									*	36	2.3
5					*								*	28	1.96
6						*							*	54	1.1
7							*						*	48	1.5
8								*					*	54	1.12
9		*	*											44	1.68
10		*			*									52	1.36
11		*					*							60	1.18
12				*	*									52	1.36
13				*			*							52	1.08
14						*	*							56	1.4
15													**	70	.84
16									*				*	42	1.8
17										*			*	60	1.0
18												*	*	22	3.44
19			*	*										56	1.3
20											*		*	6	4.2
21	*	*												34	3.6

REMARKS. Leucocytes obtained after 2 bouillon injections, only 20 ccm Exudate recovered.

Injected Guineapig Serum = Serum of the Guineapig injected with bouillon for exudate, otherwise normal.

Normal another perfectly fresh Guineapig never so injected.

EXPERIMENT No VI. 5-7-'07

No	I Normal Guineapig serum	II N. Guin. heated 60°	III 1/10 N.H.	IV 1/50 N.H.	V 1/100 N.H.	VI 1/500 N.H.	VII 1/10 N.	VIII 1/50 N.	IX 1/100 N.	X NaCl	Result: Percentage of Noughts	Bacteria p.leucocyte
1	*	*									52%	1.28
2	*		*								36	2.8
3	*			*							22	3.62
4	*				*						18	5.
5	*					*					33	4.5
6		*					*				50	1.08
7			*				*				34	2.22
8				*			*					*bad*
9					*		*				43	2.5
10						*	*				44	2.68
11		*						*			72	0.92
12			*					*			74	0.9
13				*				*			63	1.04
14					*			*			68	1.04
15						*		*			52	1.26
16		*							*		66	0.9
17			*						*			*bad*
18				*					*		42	0.9
19					*				*		56	1.4
20						*			*			
21	*									*	12	5.5
22		*								*	62	0.94
23			*							*	60	0.9
24				*						*	66	0.96
25					*					*	62	0.84
26						*				*	56	0.9
27							*			*	14	3.24
28								*		*	35	2.6
29									*	*	60	1.6

Normal Guineapig Serum is from Guineapig injected with bouillon for exudate.

EXPERIMENT No VII. 6-7-'07

No	I Leucocytes	II T. B.	III P. B.	IV Normal Guineapig	V Normal Human	VI Tuberculin 1-10,000	VII T.B. Filtrate	VIII Autolysed leucocytes	IX NaCl	X Pyocyaneus Filtrate	XI Pyocyaneus T. S.	XII P.I.S.H.60°	XIII Normal Injected Guineapig	Results Percentage of Noughts	Results Bacteria per
1	*	*							**					58%	0.88
2	*	*		*					*					16	3.48
3	*	*			*				*					50	1.57
4	*	*				*			*					60	0.98
5	*	*					*		*					90	0.2
6	*	*						*	*					60	0.76
7	*	*					*	*						88	0.24
8	*		*						**					90	0.3
9	*		*	*					*					52	0.76
10	*		*		*				*					54	0.82
11	*		*			*			*					28	1.36
12	*		*					*	*					52	0.82
13	*		*	*				*						56	0.76
14	*		*						*	*				70	0.56
15	*		*	*						*				76	0.5
16	*	*		*			*							66	1.02
17	*		*						*		*				*too many to count*
18	*		*	*								*		18	3.14
19	*	*							*		*			26	2.5
20	*		*						*			*		54	1.28
21	*		*		*							*		54	0.8
22	*	*							*				*		*bad*
23	*		*									*	*	26	3.46
24	*		*						*				*	46	0.78

Remarks, Normal Injected = Normal Guineapig injected with bouillon for leucocyte exudate. Pyocyaneus Immune Serum is from Guineapig 7th June $^1/_{20}$ ccm pyoc. Culture, 15th June $^1/_{10}$, 17th June $^1/_5$, 21st June $\frac{1}{2}$, 24th June 1 ccm.(lethal dose $\frac{1}{4}$ ccm.). P.I.S.H. = Pyocyaneus Immune Serum Heated 60°. Pyocyaneus Culture very green and viscid. Pyocyaneu specimens stained Giemsa. Autolysed Leucocytes (under toluol for 19 days) washed with NaCl and centrifuged, obtained from aseptic abscess in Dog.

EXPERIMENT No VIII. 16-7-'07

No	I Tub. Guineapig serum	II Tub. Serum 60°	III Tub. 56°	IV Normal Guineapig Serum	V N.G.S. 60°	VI N. G.S. 56°	VII N. G.S. 1/100	VIII N.G.Sheated 56° 1/100	IX Tuberculin 1-10,000	X Tub. filtrate	XI Normal human serum	XII NaCl	Results: Percentage of noughts	Results: Bacteria per leucocyte
1	*	*											51%	1.2
2	*		*										33	1.71
3	*			*									44	1.58
4	*				*								50	1.2
5	*					*							54	1.37
6	*						*						30	1.78
7	*							*					28	1.86
8	*								*				54	0.9
9	*									*			74	0.52
10	*										*		68	0.74
11	*											*	36	2.06
12		*		*									50	1.6
13		*			*								64	.66
14		*				*							76	.64
15		*					*						60	.72
16		*						*					66	.76
17		*							*				72	.66
18		*								*			90	.38
19		*									*		72	.68
20		*										*	66	.64
21			*	*									42	1.92
22			*		*								66	.56
23			*			*							64	.96
24			*				*						66	1.0
25			*					*					74	.5
26			*						*				76	.4
27			*							*			80	.26
28			*								*		84	.44
29			*									*	52	1.52
30				*	*								64	.7
31				*		*							50	2.04
32				*			*						34	2.52

EXPERIMENT No VIII (contd) 16-7-'07

No	I Tub. Guineapig serum	II Tub. Serum 60°	III Tub. 56°	IV Normal Guineapig serum	V N.G.S. 60°	VI N. G. S. 56°	VII N. G. S. 1/100	VIII N. G.S.heated 56° 1/100	IX Tuberculin 1-10,000	X Tub. filtrate	XI Normal human serum	XII NaCl	Results: Percentage of noughts	Results: Bacteria per leucocyte
33				*				*					32%	2.41
34				*					*				66	.74
35				*						*			70	.46
36				*							*		60	.76
37				*								*	24	2.8
38					*		*						64	.76
39					*			*					68	.7
40					*				*				68	.74
41					*					*			76	.5
42					*						*		70	.66
43					*							*	74	.78
44						*	*						54	1.56
45						*		*					82	1.5
46						*			*				82	.46
47						*				*			76	.14
48						*					*		68	.92
49						*						*	52	1.34
50							*					*	64	.78
51								*				*	78	.58
52									*			*	80	.38
53										*		*	84	.42
54											*	*	72	.76
55												**	82	.62

Incubation ¼ hour. Guineapig peritoneal exudate leucocytes obtained after two injections of bouillon, one 4 hours, one 18 hours before extraction. Serums heated for ½ hour. Koch's old Tuberculin used.

EXPERIMENT No IX. 24-7-'07

No	I Normal Guineapig serum	II Normal heated 56°	III Normal Guineapig heated 60°	IV Normal 1/50	V Normal Human serum	VI Normal Human 56°	VII Normal Human 60°	VIII Tub. Guineapig serum	IX NaCl	X Tub. heated 56°	XI Tub. heated 60°	Results. Percentage of noughts	Bacteria per Leucocyte
1	**											-45	
2	*								*			-24	
3		**										48%	3.86
4		*							*			50	1.18
5			**									26	2.32
6			*						*			66	0.9
7				*					*			30	2.32
8					**							38	2.2
9					*				*			62	1.08
10						**						46	1.82
11						*			*			40	1.54
12							**					30	2.02
13							*		*			44	1.42
14								**				3	7.1
15								*	*			10	5.83
16										**		16	3.28
17									*	*		34	2.0
18											**	54	2.0
19									*		*	80	0.78
20	*	*										26	3.5
21	*		*									22	2.2
22	*				*							-	-
23	*					*						74	0.82
24	*						*					55	1.5
25	*							*				46	3.46
26	*									*		-	-
27	*										*	56	1.16
28		*		*								63	2.48
29		*			*							90	.38
30		*				*						66	1.2

EXPERIMENT No IX. (Contd.) 24-7-'07

No	I Normal Guineapig serum	II Normal heated 56°	III Normal Guineapig heated 60°	IV Normal 1/50	V Normal Human serum	VI Normal Human 56°	VII Normal Human 60°	VIII Tub. Guineapig serum	IX NaCl	X Tub. heated 56°	XI Tub. heated 60°	Results. Percentage of noughts	Results. Bacteria per leucocyte
31		*						*				46%	1.6
32		*								*		55	1.0
33		*									*	-	-
34					*			*				77	0.55
35					*					*		82	0.3
36					*						*	78	0.5
37						*		*				88	0.3
38						*				*		74	0.8
39						*					*	84	0.58
40							*	*				52	1.5
41							*			*		80	0.52
42							*				*	96	0.24
43				*		*						88	0.52
44				*						*		58	1.66
45					*	*						82	0.57
46					*		*					-	-
47									**			-	-

Remarks. Date, 24th July 1907. Tub. Guineapig injected in Feb; p. m. only one gland. Guineapig leucocytes, ¼ hour incubated.

EXPERIMENT No X. 9-10-'07

No	Human Serum I	Human Serum I heated 60°	Human Serum I heated 56°	Human Serum II	Human Serum III	Human Serum IV	NaCl	Index
1	*						*	2.03
2		*					*	0.55
3			*				*	0.2
4				*			*	2.7
5					*		*	1.7
6						*	*	4.0
7							**	0.33
8	*	*						2.36
9	*		*					1.0
10	*			*				3.6
11	*				*			4.16
12	*					*		4.0
13		*			*			3.4
14			*		*			0.83
15			*			*		-

Notes. Human Serum I (non-tuberculous) Human Serum II (non-tuberculous servant) Human Serum III Tuberculous patient under treatment of injections of tuberculin. Human Serum IV, T.B. patient, supposed healed (Hall)

Incubation, ¼ hour

Human leucocytes used.

In this and all succeeding experiments the expression "Index" is used for brevity instead of "Bacteria per leucocyte" which it implies.

EXPERIMENT No XI. 11-10-'07

No	Human Serum I	Human Serum I heated 60°	Human Serum I heated 56°	Human Serum II	Human Serum III	Human Serum IV	NaCl	Index.
1	*						*	3.85
2		*					*	0.63
3			*				*	0.23
4				*			*	3.7
5					*		*	2.6
6						*	*	3.2
7							**	0.22
8	*	*						3.8
9	*		*					1.22
10	*			*				4.25
11	*				*			4.0
12	*					*		4.0
13		*			*			4.0
14			*		*			*protoplasm unstained.*
15			*			*		2.5

Serum I = A.E.P. non. Tub.
Serum II = non Tub. servant.
Serum III = Tub. patient getting tuberculin
Serum IV = Lab. Assistant healed Tubercle.
Human Leucocytes used
Incubated ¼ hour.

EXPERIMENT No XII. 16-10-'07

No	Normal Human Serum I	I heated 60°	I heated 56°	Normal Human Serum I, Acid	Normal Human Serum II	Tub. Human Serum III	Tub. Human Serum IV	NaCl	Index.
1	*							*	3.45
2		*						*	0.12
3			*					*	0.62
4				*				*	3.3
5					*			*	5.4
6						*		*	4.85
7							*	*	-
8								**	0.13
9	*	*							3.7
10	*		*						3.4
11	*			*					5.83
12	*				*				6.86
13		*		*					5.4
14		*				*			2.2
15			*	*					2.9
16			*			*			4.3

Notes. Human Leucocytes ¼ hour incubated.
Serums as before. Acid serum = 1% HNo_3 added to serum, 0.2 serum + .05 cc. Acid.

EXPERIMENT No XIII. 22-10-'07

No	Normal Human serum	I heated 60°	I heated 56°	Normal Human serum I, Acid	Normal Human serum II	NaCl	Index.
1	*					*	2.85
2		*				*	0.2
3			*			*	0.23
4				*		*	-
5					*	*	4.3
6						**	0.2
7	*	*					2.6
8	*		*				2.2
9	*			*			3.7
10	*				*		3.26
11		*	*				0.33
12		*		*			3.3
13		*			*		-
14			*	*			1.76
15			*		*		1.06

Human Leucocytes ¼ hour incubated.
Acid Serum = 0.2 cc. Serum + 0.05 cc. 1% HCl.

EXPERIMENT No XIV. 24-10-'07

No	Normal Human Serum I.	I. 1/10	I. 1/50	I heated 60°	I heated 56°	I heated 56° 1/10	I heated 56° 1/50	Normal Human Serum II	NaCl	Index
1	*								*	2.66
2		*							*	0.4
3			*						*	0.2
4				*					*	0.23
5					*				*	0.3
6						*			*	0.03
7							*		*	0.
8								*	*	3.26
9									**	0.03
10	*							*		2.7
11	*				*					1.33
12	*					*				2.5
13	*						*			2.5
14		*			*					0.33
15		*				*				0.43
16		*					*			0.43
17					*			*		1.9

Human Leucocytes. ¼ hour incubated.

EXPERIMENT No XV. 31-10-'07

No	Human Serum Normal I.	I. 1/10	I. 1/50	I. heated 60°	I. heated 56°	I. heated 56° 1/10	I. heated 56° 1/50	Tub. Human Serum IV	NaCl	Index
1	*								*	4.76
2		*							*	2.0
3			*						*	0.26
4				*					*	0.1
5					*				*	0.7
6						*			*	-
7							*		*	0.25
8								*	*	5.13
9									**	0.2
10	**									6.4
11	* *			*						4.63
12	* *				*					2.66
13	* *					*				3.83
14	* *						*			4.8
15	*							*		5.3
16		*			*					1.65
17						*		*		1.4
18					**					0.7
19					*			*		2.4
20				**						0.2

Notes. Human Leucocytes ¼ hour incubated. Serums I and IV as in Experiment I.

EXPERIMENT No XVI. 4-11-'07

No	Normal Human Serum I.	I. 1/10	I 1/50	I. heated 60°	I. heated 56°	I. 56° 1/10	Normal Human Serum II.	Tub. Human III	Tub. Human IV	Tub. Human V	NaCl	Index.
1	*											4.66
2							*					4.13
3								*				3.4
4									*			2.66
5										*		2.43
6	*										*	4.76
7		*									*	2.1
8			*								*	0.9
9				*							*	0.2
10					*						*	0.53
11						*					*	0.2
12											**	0.1
13	**											5.33
14	*			*								4.8
15	*				*							3.76
16	*					*						4.2
17	*						*					5.16
18		*			*							1.7
19		*				*						2.0
20					**							0.56
21					*		*					3.7
22							*				*	5.26

Remarks. Human Leucocytes. Incubated ¼ hour. Serums III, IV, and V, tuberculous patients after Vacc: with Beraneck's Tuberculin. Heating = ½ hour for 56° and 60°.

EXPERIMENT No XVII. 11-11-'07

No	Normal Human Serum I.	I. 1/10	I. heated 60°	I. heated 56°	I. 56° 1/10	Normal Human Serum II	Tub. Human III	Tub. Human IV	Tub. Human V	Normal Guineapig Serum	NaCl.	Index.
1	*											4.13
2						*						3.66
3							*					3.0
4								*				3.83
5									*			3.73
6	*										*	4.2
7		*									*	2.5
8			*								*	-
9				*							*	0.5
10					*						*	0.4
11										*	*	3.66
12											**	0.2
13	*									*		1.8
14	*		*									-
15	*			*								2.06
16	*				*							2.75
17	**											4.43
18		*		*								1.23
19		*			*							1.73
20				*						*		2.8

Remarks Human Leucocytes. Incubated ¼ hour.
Serums III, IV, V, Tub. serums, same as in last experiment.
Serums heated ½ hour.

EXPERIMENT No XVIII. 12-11-'07

No	Normal Human Serum I	Normal Human Serum II	Tub. Human Serum III	Tub. Human Serum IV	Tub. Human Serum V	Guineapig Normal Serum	Index
1	*						4.3
2		*					3.8
3			*				3.9
4				*			3.8
5					*		3.86
6						*	3.6
7	*	*					6.43
8	*					*	2.03

Remarks. Human Leucocytes. ¼ hour incubated.

Serums III, IV and V as in last Experiment.

EXPERIMENT No XIX. 14-11-'07

No	Tub. Human Serum IV	IV 1/10	IV heated 60°	IV heated 56°	IV, 56° 1/10	Normal Human Serum I	NaCl	Index.
1	*						* *	5.6
2		*					* *	4.1
3			*				* *	-
4				*			* *	0.56
5					*		* *	0.3
6						*	* *	5.7
7							**	0.26
8	**							5.93
9	*		*					3.76
10	*			*				1.0
11	*				*			3.23
12	*					*		6.0
13		*		*				2.5
14		*			*			0.7
15			**					0.2
16				**				0.6
17				*		*		2.0
18			*			*		3.2

Remarks. Human Leucocytes. Incubated ¼ hour.

Heated Serums for ½ hour.

EXPERIMENT No XX. 30-12-'07

No	Tub. Human Serum I	I heated 60°	I heated 56°	Tub. Human Serum II	II heated 60°	II heated 56°	Normal Human Serum III	III heated 60°	III heated 56°	NaCl	Index
1	*									*	3.5
2		*								*	0.2
3			*							*	0.4
4				*						*	3.23
5					*					*	0.2
6						*				*	0.56
7							*			*	3.56
8								*		*	0.33
9									*	*	0.23
10										**	0.2
11	*	*									3.36
12	*		*								1.4
13	*			*							4.13
14				*	*						3.2
15				*		*					1.83
16							*	*			3.4
17							*		*		2.7
18	*								*		2.5

Remarks. Tub. Serum I of moderately bad case, doing badly lately. Tub. Serum II of moderately bad case, but rather better than Serum I, vaccinated with Beraneck's Tub., three weeks previously. Incubated ¼ hour. Human Leucocytes. Heated Serum ½ hour at 56° and 60°.

EXPERIMENT No XXI. 2-1-'08

No	TuB. Human Serum I	I heated 56°	TuB. Human Serum II	II heated 56°	Normal Human Serum III	III heated56°	Normal Human Serum IV	IV heated 56°	NaCl	Index
1	*								*	5.16
2		*							*	0.3
3			*						*	3.76
4				*					*	0.4
5					*				*	4.36
6						*			*	0.5
7							*		*	5.0
8								*	*	0.4
9									**	0.2
10	*	*								2.5
11	*			*						2.33
12	*					*				4.44
13	*							*		4.33
14			*	*						2.23
15		*	*							2.23
16			*			*				3.06
17			*					*		3.2
18					*	*				3.73
19		*			*					2.43
20				*	*					2.53
21					*			*		3.63
22							*	*		4.23
23							*			2.66
24				*			*			2.53
25						*	*			4.0

Remarks. Human Leucocytes. ¼ hour incubated

TuB. Serum I, moderately severe case, female patient.

TuB. Serum II, moderately severe, female patient, injected with Beraneck's T.B. one hour before blood taken.

EXPERIMENT No XXII. 3-1-'08

No	Tub. Human Serum I old	I (old) heated 56°	Normal Human Serum III fresh	Normal Human Serum III old	III (old) heated 56°	NaCl	Index
1	*					*	1.16
2		*				*	0.2
3			*			*	1.6
4				*		*	1.23
5					*	*	0.23
6		*	*				0.83
7			*		*		1.33
8	*	*					0.7
9		*		*			0.7

Remarks. Serums I (old) and III (old) = same specimens as of No XXI, but kept for 24 hours. Human Leucocytes used. ½ hour incubated. Fresh serum from same source as III to compare.

EXPERIMENT No XXIII 9-1-'08

No.	Untreated Tub. Culture	Tub. treated at 1° C. excess of Normal Human Serum	Normal Human Serum I.	Normal Serum I heated 56°	Normal Human treated ā Tub. at 1° in excess I_A	Normal Human treated ā Tub. excess of Serum I_B	Tub. Human Serum II	Tub. Serum heated 56° II	NaCl.	Index.
1	*		*						*	2.65
2	*			*					*	0.13
3	*				*				*	0.16
4	*					*			*	0.3
5	*						*		*	1.7
6	*							*	*	0.1
7	*								**	0.1
8	*		*	*						1.2
9	*		*		*					0.9
10	*		*					*		1.0
11		*	*						*	0.4
12	*			*	*					0.7
13	*			*			*			1.0
14		*						*	*	0.03
15	*						*	*		0.56
16		*								0.1

Remarks. Human Leucocytes. Serum heated at 56° for ½ hour, and the Serum treated at 1° C. also ½ hour.

EXPERIMENT XXIV. 21-1-'08

No	Normal Human Serum I	Tub. Human Serum II	Tub. Human Serum III	I 56°	II 56°	III 56°	NaCl.	Index
1	*						*	3.5
2		*					*	2.6
3			*				*	4.0
4				*			*	0.35
5					*		*	1.1
6						*	*	0.15
7	*			*				2.8
8		*			*			2.4
9			*			*		2.36
10							**	0.1

Remarks. Human Leucocytes. Incubated ¼ hour.

Serum heated to 56° for ½ hour

EXPERIMENT No XXV. 1-2-'08

No	Tub. Serum Original	Tub. Serum heated 56°	Tub. Serum 56° 1/10	Tub. Serum 56° 1/100	From Exterior of Collod: Sac after one hour's dialysis. A	From ext: of Collod: Sac. B.	Mixture of Orig: and same 56°	Mixture of Orig: and 1/10 56°	Mixture of Orig: and 1/100 56°	Mixture of Orig: and 1/500 56°	NaCl.	Index.		
1	*										*	116	3.86	
2	*	*										81	2.7	
3	*		*									61	2.03	
4	*			*								118	3.93	
5							**					31	1.03	
6								**				99	3.3	
7									**			80	2.66	
8										**		115	3.83	
9	*				*							96	3.2	
10	*					*						76	2.53	
11					*						*	0.06	0.06	
12											**	0.06	0.06	

Remarks Human Leucocytes. Incubated ¼ hour
Tuberculous Human Serum was taken, a part left as Control, a second part placed in a Collodion sac in an equal quantity of saline solution in a glass tube, a third part heated to 56° for ½ hour. The heated serum was diluted to different strengths, 1-10th, 1-100th and 1-500th. Then mixtures were made of equal parts original unheated + each strength of heated and heated-diluted. These mixtures were left overnight, some nineteen hours. A part of heated 56° undiluted was placed in another Collodion sac, as in the case of a part of the original unheated serum. After each had been subjected to one hour's dialysis, a portion of the saline exterior to each Collodion sac was taken out, and kept till experiment.

EXPERIMENT No XXVI. 3-2-'08

No	A Tub. Human Serum 2 days old Control	B Tub: Serum dialysed through Collodion after two days	C Mixture of A & B after ¼ hour	D Tuberculous Serum heated 56°, 2 days old	E Mixture of A & D, after ¼ hour	NaCl.	Index
1	*					*	2.66
2	*	*					1.9
3			**				0.9
4	*			*			1.43
5					**		0.36
6						**	0.03

REMARKS. Human Leucocytes. Incubation ¼ hour
Tube of Collodion containing 0.25 cc. Tub. Serum was placed in a larger glass tube containing equal quantity of physiological saline solution.

EXPERIMENT XXVI a. 5-2-'08

No	Normal Human Serum I	I 56°	I 56° 1/10	I treated with Bacteria at 56°	Bacteria treated with Serum at 56°	NaCl.	Index
1	*					*	2.8
2	*	*					1.5
3	*		*				2.0
4	*			*			2.1
5					*	**	0.6
6	*				*	*	1.86
7			*			*	0.23
8						**	0.13

Remarks. Human leucocytes. Incubated ¼ hour.
A portion of Human Serum was heated to 56°,
another heated at 56° for one minute then Bacteria
added in thick emulsion and kept at 56° for ½ hour.

EXPERIMENT No. XXVI b. 6-2-'08

No	Normal Human Serum Control	Normal H.S. heated 56°	N.H.S. treated with Bacteria at 37°	Salt Solution dialysed through Collodion	N.H.S. in Collodion Sac Ext: 20 m.	Tub: H.S. in Collodion Sac Ext: 20 m	N.H.S. in Collodion sac Int: 20 m.	Tub. H.S. in Collodion Sac Int: 20 m.	N.H.S. in Collodion Sac Ext: 40 m.	Tub: Human Serum in Coll: Sac Ext: 40 m.	N.H.S. in C.S. Int: 40 m.	Tub: H.S. in C.S. Int: 40 m.	N.H.S. in C.S. Int: 2½ hours	Tub. H.S. in C.S. Int 2½ hours	NaCl.	Index
1	* *														*	1.93
2	*	*														0.86
3			* *												*	0.26
4	*		*													2.06
5	* *			*												1.96
6	* *				*											1.66
7	*					*										1.83
8							*								* *	1.66
9								*							*	1.6
10	*								*							1.16
11	*									*						1.33
12											*				* *	1.13
13												*			* *	0.83
14													*		* *	0.93
15														*	* *	0.73

Remarks. Human Leucocytes. Incubated ¼ hour. Normal Human Serum treated & Bacteria at 37° for ½ hour. Serum heated 56° also ½ hour. Salt solution dialysed through Collodion 2¼ hours. Tuberculous Serum early case, several times injected with Tuberculin, index at present being generally above normal.

EXPERIMENT No XXVII. 7-2-'08

No	Normal Human Serum	Mixture equal parts N.H.S. & same heated 56°	Mixture equal parts N.H.S. + 1/10 56°	Mixture equal parts N.H.S. + 1/100 56°	Mixture NaCl Ext: of Collodion Sac + N.H.S. equal parts.	Serum from Int: of Coll: Sac.	NaCl	Index.
1	*						*	1.53
2		**						0.8
3			**					1.4
4				**				1.46
5					**			0.43
6						*	*	0.44
7							**	0.01
8 (salt solution--outside of Collodion sac, alone)							*	0.0

Remarks. Human Leucocytes. Incubated ¼ hour. Tuberculous Serum placed in Collodion Sac six days previously, and Collodion Sac put in larger glass tube containing equal quantity of physiological salt solution; a portion taken from the salt solution exterior to the sac and mixed with equal amount of 19 hours old Normal Human Serum and allowed to stand ¼ hour before using.

The mixtures of Normal Human Serum with the various strengths of heated 56° Serum had been allowed to stand over night.

EXPERIMENT No XXVIII. 11-2-'08

No	Normal Human Serum I	Normal Human Serum II	Tub. Human Serum III	Tub. Human Serum IV	Tub. Human Serum V	I kept at -1.5° for ½ hour.	II kept at -1.5° for ½ hour.	III kept at -1.5°	IV kept at -1.5°	V kept at -1.5°	Dialysed Exterior NaCl.	NaCl.	Index
1	*											*	3.46
2		*										*	3.2
3			*									*	2.96
4				*								*	3.83
5					*							*	3.03
6	*					*							1.7
7		*					*						1.43
8			*					*					1.83
9				*					*				1.7
10					*					*			2.8
11	*										*		1.33
12											*	*	0.1
13												**	0.06

Remarks. Human Leucocytes. Incubated ¼ hour
Five specimens of Serum were taken, and each separated into two portions. One of these portions was then placed in a mixture of ice and K_2SO_4. A Collodion sac containing Serum I had been made 36 hours before, placed in equal quantity NaCl solution in a glass tube. A portion taken from NaCl solution exterior to Collodion sac.

EXPERIMENT No XXIX. 16-2-'07 16-2-'08

No	Normal Human Serum I	Normal Human Serum II	Normal Human Serum III	III + T.B.Filtrate mixed for ½ hour	Serum from Interior of Collodion sac after 6 Days Dialysis.	T.B. filtrate	NaCl	Ordinary Leucocytes	Leucocytes treated with Filtrate.	Index
1	*						*	*		3.5
2		*					*	*		5.5
3			*				*	*		4.25
4				**				*		2.85
5					*		*	*		0.07
6		*				*		*		2.1
7							**	*		0.1
8	*								*	1.93
9		*							*	2.1

Remarks. Human Leucocytes. Incubated ¼ hour. Two equal portions of blood were received into Sodium Citrate, and washed with NaCl, then one of them was treated with excess of T.B. Culture Filtrate for ½ hour, then twice washed with NaCl, the other portion of blood being also washed an equal number of times (4 in all). T.B.Filtrate + equal quantity of Serum III added together and left to stand for ½ hour previous to experiment.

EXPERIMENT No XXX. 17-2-'07.

No	Normal Human Serum I	Normal Human Serum II	Normal Human Serum III	Normal Human Serum II 24 hours old.	A II from interior of Coll: sac after ¼ hour dialysis	B NaCl outside same sac after ¼ hour + II fresh.	C II from Collodion sac, after 19 hours	D NaCl outside same after 19 hours + II	E T.B. Culture Filtrate + III	NaCl	Index
1	*									*	2.16
2		*								*	2.46
3			*							*	2.8
4				*						*	2.0
5					*					*	2.13
6						**					1.6
7							*			*	1.26
8								**			1.16
9									**		1.53
10										**	0.1

Remarks Human Leucocytes. Incubated ¼ hour.

Normal Serum II, a portion put in a reed tube which was placed in a glass tube containing an equal quantity of sterile NaCl solution, control portion kept to compare.

A = a sample of the contents of the reed tube after ¾ hour

C = do do 19 hours

B = do glass tube (the filtrate) after ¾ hrs.

D = do do 19 hrs.

E = Tub. Culture Filtrate + equal quantity of Serum III, (same as on previous day which had reduced the count for the serum alone from 4.25 to 2.85) + equal quantity of fresh Serum III, standing for ½ hour

EXPERIMENT No XXXI. 19-2-'08.

No	Normal Human Serum I.	Normal Human Serum II.	Dialysed N.H.S. Int: of Reed Bag.	NaCl Ext: of same.	NaCl	Index
1	*				*	1.06
2		*			*	0.8
3			*		*	1.13
4				*	*	0.0
5	*			*		0.36
6					**	0.03

Remarks. Human Leucocytes. Incubated ¼ hour

Dialysis 2½ days duration, Serum II.

EXPERIMENT XXXII. 26-2-'08

No	Normal Human Serum I	Tub: Human Serum II	I 56°	II 56°	T.B. Culture Filtrate	NaCl	Index
1	*					*	1.83
2	*		*				1.26
3		*				*	1.2
4		*		*			0.7
5	*				*		1.5
6	*			*			1.3
7						**	0.03

Remarks. Human Leucocytes. Incubated ¼ hour

Serum heated ½ hour at 56°

EXPERIMENT No XXXIII 28-2-'08

No	Normal Human Serum I	Normal Human Serum II	Tub: Human Serum III	Tub: Human Serum IV	Tub: Human Serum V	I. 0°	II 0°	III 0°	IV. 0°	V. 0°	I treated with Bacteria at 0°	Bacteria treated with Serum I. 0°	NaCl.	Index
1	*												*	1.4
2		*											*	0.93
3			*										*	1.1
4				*									*	0.8
5					*								*	1.3
6						*							*	0.35
7	*					*								0.6
8		*					*							0.5
9			*					*						0.4
10				*					*					0.43
11					*					*				0.33
12											*		*	0.25
13	*										*			0.7
14												*	*	0.83
15	*											*		2.83
16		*										*		2.16

Remarks. Human Leucocytes. Incubated ¼ hour

Five specimens of Serum were taken and divided into two portions, one left as Control, the other put in ice and kept at 0° C. for ½ hour.

Bacteria were ground and placed in excess of Serum I at 0°, and kept at 0° for ½ hour, then centrifuged, separated from the Serum, washed twice with 1-1000 saline, and then made into an emulsion which was unfortunately thicker than the emulsion of untreated Bacteria.

EXPERIMENT No XXXIV. 2-3-'08.

No	Normal Human Serum Control, 40 hrs old	Normal Serum Coll: Ext: 20 min.	N. Serum Reed Ext: 20 minutes	N. Serum 0° Reed Ext: 20 minutes.	N. Serum Coll: Ext: 40 minutes.	N. Serum Reed Ext: 40 minutes	N. Serum 0° Reed Ext: 40 minutes	N. Serum Coll: Int: 60 minutes.	N. Serum Reed Int: 60 minutes.	N. Serum Collodion Ext: 60 minutes.	N. Serum Reed Ext: 60 minutes.	N. Serum Collodion Int: 40 hours.	N. Serum Reed Int: 40 hours.	NaCl.	Index
1	*													*	1.13
2	*	*													0.5
3	*		*												-
4	*			*											0.16
5	*				*										0.2
6	*					*									0.5
7	*						*								0.23
8								*						*	0.4
9									*					*	0.9
10	*									*					0.16
11	*										*				0.5
12												*		*	0.23
13													*	*	0.96
14														**	0.0

Remarks. Human Leucocytes. Incubated ¼ hour.
A specimen of Normal Human Serum was taken and divided into four equal portions, one kept as Control, one placed in Collodion, one in a Reed tube, the fourth cooled at 0° for ½ hour and placed in another Reed sac. These sacs were each placed in larger glass tubes containing an equal quantity of NaCl solution. Portions of the NaCl solution were taken out at the end of 20, 40, and 60 minutes respectively, and kept separately till experiment 40 hours later

EXPERIMENT No XXXV. 4-3-'08.

No	Normal Human Serum	Same Serum treated with Gelatine	Same Serum treated with Starch.	Same Serum treated with Collodion	Same Serum treated with Leucocytes at 10-15°	Leucocytes treated with Serum at 10-15°	NaCl.	Index
1	*						*	1.1
2	*	*						1.2
3	*		*					1.66
4	*			*				0.7
5					*		*	0.9
6						*	**	0.13
7							**	0.0

Remarks. Human Leucocytes. Incubated ¼ hour
Normal Human Serum was taken and divided into five parts, one kept as Control, into a second a piece of gelatine soaked in NaCl solution, into a third a piece of stiff boiled starch, into a fourth a piece of collodion, and these were left over night.
Next morning Leucocytes were added to the fifth and after ½ hour separated by centrifuging, washing etc.

EXPERIMENT No XXXVI. 5.3.'08.

No	Normal Human Serum	N.H.S. heated 56° for ½ hour.	N.H.S. 1-10th heated 56° for ½ hour	N.H.S. 56° treated with Bacteria for ½ hour	Tubercle Bacteria treated with N.H.S. 56° for ½ hour	NaCl., 0.85%	Index.
1	*					*	2.8
2		*				*	0.23
3				*		*	0.1
4					*	*	0.6
5	*	*					1.5
6	*		*				2.0
7	*			*			2.1
8	*				*		1.85
9						**	0.06

Remarks. Human Leucocytes. Incubated ½ hour

EXPERIMENT No XXXVII. 6-3-'08

No	Normal Human Serum I Control	Tub: Human Serum II, Control.	I heated 56° for ½ hour.	I. treated at 37° for ½ hour with Bacteria.	Saline Solution in which Collodion has been standing overnight.	I. Ext: Collodion 20 minutes.	II. Ext. Collodion 20 minutes.	I. Int: 20 min.	II. Int: 20 min.	I. Ext: 40 min.	II. Ext: 40 min.	I. Int: 40 min.	II. Int: 40 min.	I Int: 2¾ hours.	II. Int: 2¾ hours	NaCl.	Index.
1	*															*	1.96
2		*														*	2.2
3	*		*														0.86
4				* *												*	0.26
5	*			*													2.03
6	*				*												1.99
7	*					*											1.66
8	*						*										1.83
9								*								*	1.66
10									*							*	1.6
11	*									*							1.33
12	*										*						1.16
13												*				*	1.13
14													*			*	0.83
15														*		*	0.93
16															*	*	0.73
17																**	0.03

Remarks. Human Leucocytes. Incubated ¼ hour

EXPERIMENT No XXXVIII. 14-3-'08.

No.	Normal Guineapig Serum I.	Normal Human Serum II	Normal Human Serum III	I. heated 60°	II. heated 56°	III. heated 56°	I. cooled at 0°	II. cooled at 0°	III. cooled at 0°	NaCl, 0.85%	Index.
1	*									*	1.12
2		*								*	1.66
3			*							*	1.0
4				*						*	0.1
5					*					*	0.0
6						*				*	0.23
7							*			*	1.2
8								*		*	0.6
9									*	*	0.4
10										**	0.0
11	*						*				1.03
12		*						*			0.83
13			*						*		1.0
14	*	*									0.75
15	*		*								0.7
16		*	*								1.7

Remarks. Human Leucocytes. Incubated ¼ hour

Heating and cooling of Serum ½ hour

EXPERIMENT No XXXIX. 24-3-'08

No	Normal Human Serum, 30 hours old	Same dialysed ¼ hour.	Ext:¼ hour	Int:2½ hours	Ext: 2½ hours	Int: 4 hours	Ext: 4 hours	Int: 6 hours	Ext: 6 hours	Int: 22 hours	Ext: 22 hours	Int: 30 hours	Ext: 30 hours	NaCl	Index
1	*													*	5.18
2		*												*	4.83
3			*											*	0.13
4				*										*	1.93
5					*									*	0.1
6						*								*	1.1
7							*							*	0.1
8								*						*	0.7
9									*					*	0.1
10										*				*	0.33
11											*			*	0.13
12												*		*	0.21
13													*	*	0.06
14	*		*												4.98
15	*				*										3.5
16	*						*								3.02
17	*								*						2.7
18	*										*				2.75
19	*												*		2.5
20	**														7.0
21	*											*			4.9
22														**	0.1
23										*	*				0.3
24												*	*		0.25

Remarks. Human Leucocytes ¼ hour incubated.
"Int:" means Serum inside dialysing sac removed at intervals and kept till experiment."Ext:" means NaCl external to sac. Collodion sac.

EXPERIMENT No XL. 25-3-'08.

No	Normal Human Serum I A.E.P.	N.H.S. Hink II	N.H.S.Dr Kamm III	N.H.S.Dr Gachtgen IV	N.H.S., Bettiger V	N.H.S. Dr Müller vi	I 0°	II 0°	III 0°	IV 0°	V 0°	VI 0°	Control Normal Human Serum, 2 days old	Same dialysed for 2 days	NaCl Ext: of Collodion Sac	NaCl	Index
1	*															*	2.98
2		*														*	2.0
3			*													*	3.0
4				*												*	2.5
5					*											*	2.0
6						*										*	3.0
7							*									*	2.33
8								*								*	1.7
9									*							*	2.53
10										*						*	2.5
11											*					*	2.1
12												*				*	2.63
13	*						*										3.0
14		*						*									1.73
15			*						*								3.1
16				*						*							2.6
17					*						*						2.83
18						*						*					1.5
19													*			*	2.2
20														* *		*	0.4
21	*													* *			2.7
22														*	*		0.23
23	*														*		0.7
24																**	0.2

Remarks. Human Leucocytes. ¼ hour incubated

EXPERIMENT No XLI. 26-3-'08

No	Normal Guineapig Serum	Normal Guineapig Serum 1-5th diluted in NaCl.	1-5th N.G.S. treated with T.B. at 37° filtered heated 60	1-5th N.G.S. kept at 37° filtered heated 60°	1-5th N.G.S. treated at 37° filtered	1-5th N.G.S. kept at 37° filtered	1-5th N.G.S. treated with T.B. at 0° filtered	1-5th N.G.S. kept at 0° & filtered.	T.B. treated with serum 1-5th at 37°	T.B. treated with 1-5th Serum at 0°	NaCl	Index.
1	*										*	8.93
2	*								*		*	5.0
3	*									*	*	1.42
4		*									*	5.7
5		*							*		*	2.2
6		*								*	*	0.7
7			*								*	0.1
8				*							*	0.5
9							*				*	*no leucocytes*
10								*			*	*no leucocytes*
11		*	*									0.1
12		*		*								2.6
13		*					*					0.3
14		*						*				3.66
15		*				*						0.06
16		*				*						4.62
17											**	0.18
18		**										
19									*		**	0.05
20										*	**	0.05

Remarks. Human leucocytes. Incubation ½ hour.

Bacterial emulsions made far too thin.

EXPERIMENT No XLII. 27-8-'08.

No	1-5th N.G.S.	1-5th N.G.S. absorbed with T.B. 37°	1-5th N.G.S. kept at 37° filtered.	1-5th N.G.S. absorbed with T.B. at 0°	1-5th N.G.S. kept at 0° filtered.	Fresh 1-5th kept at 37°	Fresh 1-5th N.G.S. filtered kept at 37°	Fresh 1-5th N.G.S. with T.B. at 37° filtered	NaCl.	T.B.	Typhoid.	T.B. absorbed in 1-5th N.G.Serum	Ty absorbed in 1-5th N.G.S.	Index	
1	*									*				1.8	
2	*										*			0.6	
3	*											*			0.1
4	*												*	2.8	
5		*								*				0.06	
6		*									*			0.0	
7		*										*			0.0
8		*											*	0.2	
9			*							*				0.45	
10			*								*			0.02	
11			*									*			0.13
12			*										*	1.0	
13				*						*				0.05	
14				*							*			0.1	
15				*								*			0.1
16				*									*	0.0	
17					*					*				0.08	
18					*						*			0.4	
19					*							*			0.22
20					*								*	1.7	
21						*				*				2.6	
22						*					*			0.7	
23						*						*			0.32
24						*							*	0.2	

Remarks. Human Leucocytes. ¼ hour incubated.

Treated T.B. Culture far too thin.

(Continued)

EXPERIMENT No XLII (Contd) 27-3-'08

No	1-5th N.G.S.	1-5th N.G.S. absorbed with T.B. 37°	1-5th N.G.S. kept at 37° filtered.	1-5th N.G.S. absorbed with T.B. at 0°	1-5th N.G.S. kept at 0° filtered	Fresh 1-5th N.G.S. kept at 37°	Fresh 1-5th N.G.S. kept at 37° filtered	Fresh 1-5th N.G.S. with Ty at 37° filtered.	NaCl.	T.B.	Ty	T.B. absorbed in 1-5th N.G.Serum.	Ty absorbed in 1-5th N.G.S.	Index.	
25							*			*				0.6	
26							*				*			0.2	
27							*					*			0.2
28							*						*	0.8	
29								*		*				0.1	
30								*			*			0.0	
31								*				*			0.02
32								*					*	1.0	
33									*	*				0.1	
34									*		*			0.3	
35									*			*			0.02
36									*				*	1.1	

Remarks. Human Leucocytes. ¼ hour incubated.

Ty = Typhoid. T.B. = Tubercle Bacteria.

EXPERIMENT No XLIII. 27-3-'08.

No	Normal			Guineapig Serum							Index
	1/50	1/100	1/500	1/800	1/1000	1/2000	1/5000	1/8000	1/10,000	0.85% NaCl	
1	*										5.0
2		*									5.26
3			*								3.0
4				*							3.92
5					*						3.5
6						*					4.08
7							*				2.93
8								*			3.08
9									*		3.1
10										*	1.8

Remarks. Human Leucocytes. ¼ hour incubated.

Dilutions made with 0.85% NaCl.

EXPERIMENT No XLIV. 9-3-'08.

No	N. H. S. I.	N. H. S. II.	N.H.S. I. treated with filter paper.	N.H.S. I. heated 56°	N.H.S. I. treated with filter paper heated 56°	N.H.S. I Acid.	N.H.S. II. Acid.	Leucocytes treated with 56° heated Serum	Bacteria treated with 56° Serum	0.85% NaCl.	Index.
1	*									*	10.0
2		*								*	9.0
3			*							*	4.73
4				*						*	0.8
5					*					*	0.6
6						*				*	0.53
7							**				0.2
8									*	**	0.36
9	*			*							7.0
10	*				*						4.26
11	*					*					10.8
12	*							*			8.3
13	*								*		5.86
14										**	0.46

Remarks. Human Leucocytes. ¼ hour incubated. Acid Serum, equal parts serum and $\frac{N}{3}$ HCl standing together for ½ hour before experiment. Treatment with Filter Paper or Heating at 56°, ½ hour.

EXPERIMENT No XLV. 11-4-'08.

No	N. H. S.	N.H.S. + $\frac{N}{3}$ HCl equal parts	N.H.S. + $\frac{N}{3}$ HCl 4 to 1	N.H.S. + $\frac{N}{6}$ HCl 4-1	N.H.S. + $\frac{N}{15}$ HCl 4-1	N.H.S + $\frac{N}{30}$ HCl 4-1	N.H.S. + $\frac{N}{150}$ HCl 4-1	N.H.S. + $\frac{N}{300}$ HCl 4-1	N.H.S. dialysed for 2 days.	N.H.S. Control 2 days old.	0.85% NaCl	Index.
1	*										*	5.0
2		**										0.43
3			**									4.0
4				**								4.3
5					**							5.0
6						**						4.66
7							**					5.66
8								**				4.5
9									*		*	1.4
10										*	*	4.8
11											**	0.33
12	*	*										1.3
13	*		*									5.4
14	*			*								7.0
15	*								*			6.0

Remarks. Human Leucocytes. $\frac{1}{4}$ hour incubated. Collodion sac used for dialysing. Acid Serum treated for $\frac{1}{4}$ hour

EXPERIMENT No XLVI 13-4-'08.

No	N. H. S.	N. H. S. heated 51°	N. H. S. 53°	N. H. S. 54.5°	N. H. S. 56.5°	N. H. S. 58°	N. H. S. 60.5°	Dialysed N.H.S. 4 days old. heated 50	Dialysed N.H.S. 4 days old.	Control N.H.S. 4 days old.	5 hours old Dialysed Serum (NaCl outside in excess and renewed).	Dialysed Serum 5 hours old standing in 0.3 cc NaCl.	N.H.S. + $\frac{N}{2}$ HCl for ½ hour, then $\frac{N}{2}$ NaOH equal parts 1-1-1	0.85% NaCl	Index
1	*													*	4.0
2		*												*	2.3
3			*											*	1.35
4				*										*	1.06
5					*									*	1.0
6						*								*	0.8
7							*							*	1.0
8								*						*	1.8
9									*					*	1.0
10										*				*	3.5
11											*			*	1.82
12												*		*	1.72
13													*	*	2.1
14														**	0.6
15	*			*											3.8
16	*				*										3.5
17	*					*									3.06
18	*						*								3.9
19	*							*							3.05
20	*								*						2.2

Human Leucocytes. ¼ hour incubated.

EXPERIMENT No XLVII. 14-4-'08.

No	N. H. S.	N.H.S. 1 day old	N.H.S. 5 days old	N.H.S. dialysed 5 days	N.H.S. dialysed 1 day	N.H.S. dialysed 1 day against unlimited NaCl.	N.H.S. heated 56°	N.H.S. dialysed 5 days heated 56°	N.H.S. heated 51.5°	N.H.S. heated 53°	NaCl Exterior to dialysis sac 5 days	N.H.S.+ $\frac{N}{2}$ HCl for ½ hour then + $\frac{N}{5}$ NaOH, 1-1-1.	0.85% NaCl	Index.
1	*												*	2.78
2		*											*	2.5
3			*										*	2.32
4				*									*	0.13
5					*								*	0.8
6						*							*	0.3
7							*						*	0.2
8								*					*	0.16
9									*				*	2.06
10										*			*	0.9
11											*		*	0.13
12												**		1.5
13													*	0.1
14	*			*										1.82
15	*					*								2.2
16	*						*							2.23
17	*							*						1.4
18	*								*					0.7
19	**													3.3
20				*						*				0.26

Remarks. Human leucocytes. ¼ hour incubated.

Dialysing sac collodion. Serums heated 56° for ½ hour

EXPERIMENT No XLVIII. 16-4-'08.

No	N. H. S.	N.H.S. heated 56°	N.H.S. 7 days old	N.H.S. dialysed 7 days.	N.H.S. dialysed 3 days (NaCl unlimited)	N.H.S. dialysed 3 days	N.H.S. dialysed 7 days, heated 56°	N.H.S. dialysed 3 days, heated 56°	N.H.S. + $\frac{N}{2}$ HCl for ½ hour then $\frac{N}{2}$ NaOH 1-1-1.	N.H.S. heated 56° then kept at 37°	N.H.S. heated 53°	0.85% NaCl	Index
1	*											*	3.0
2		*										*	0.3
3			*									*	1.8
4				*								*	0.1
5					*							*	0.26
6						*						*	0.22
7							*					*	0.1
8								*				*	0.16
9									**				1.0
10										*		*	0.5
11											*	*	0.4
12												**	0.26
13	*	*											2.9
14	*			*									3.2
15	*				*								2.6
16	*					*							2.9
17	*						*						2.3
18	*							*					2.9
19	*									*			2.9
20	*										*		2.96
21	**												4.8
22	*			*									3.22

Remarks. Human leucocytes. ¼ hour incubated.

Serums heated for ½ hour.

Collodion dialysing sacs

EXPERIMENT No XLIX. 17-4-'08.

No	Normal Human Serum I	Normal Human Serum II	Tub: Human Serum III	Tub: Human Serum IV.	I. -2° left at 10-15° for 4 hours.	I. -2° 4 hours, afterwards at 37°	II. -2° 4 hours at 10-15°	III. -2° 4 hours 10-15°	IV. -2° 4 hours 10-15°	N.H.S. I. 56° for ½ hour	N.H.S. dialysed 8 days old	N.H.S. dialysed 4 days old.	N.H.S. dialysed 4 days old	Bacteria treated with N.H.Serum I at -2°	NaCl.	Index.
1	*														*	2.94
2		*													*	3.4
3			*												*	3.43
4				*											*	2.7
5					*										*	2.36
6						*									*	2.9
7							*								*	2.0
8								*							*	2.2
9									*						*	1.95
10										*					*	0.16
11	*				*											2.8
12	*					*										2.2
13		*					*									2.0
14			*					*								2.5
15				*					*							1.82
16	*										*					1.9
17	*											*				2.2
18	*												*			-
19															**	0.1
20														*	**	0.0
21	*													*	*	2.3

Remarks. Human leucocytes. ¼ hour incubated.
Serum left at -2° in ice and K_2SO_4 for ½ hour.
Bacteria and Serum at -2° for 2' before added together, centrifuged in ice-mixture.

EXPERIMENT No L. 21-4-'08.

No	N. H. S. I.	N. H. S. II.	Tub: H. S. III.	Tub: H. S. IV.	N.H.S. I. heated 56°	I. cooled -1°	II. -1°	III -1°	IV. -1°	I. treated with Bacteria at -1°	II. treated with Bacteria at -1°	Tubercle Bacteria treated with N.H.S. I. at -1°	Control Serum 5 days old.	N.H.S. dialysed 5 days.	0.85% NaCl	Index.
1	*														*	2.85
2		*													*	3.07
3			*												*	3.3
4				*											*	3.26
5					*										*	0.2
6						*									*	2.0
7							*								*	2.47
8								*							*	2.0
9									*						*	2.36
10										*					*	1.44
11											*				*	1.4
12												*			*	0.2
13													*		*	3.26
14														*	*	0.1
15															**	0.2
16	*				*											2.06
17	*					*										3.8
18	*									*						2.0
19	*											*				2.45
20	*													*		1.96
21		*					*									2.5
22			*					*								2.53
23					*					*						1.5
24					*								*			1.6
25					*	*										2.6

EXPERIMENT No LI. 23! 4-'08.

No	Normal Human Serum I	Tub: Human Serum II	I heated for ½ hour 56°	I cooled for ½ hour to -1°	II. cooled for ½ hour to -1°	I. treated with T.B. for ½ hour at -1°	II. treated with T.B. for ½ hour at -1°	T.B. treated for ½ hr with Serum I at -1°	NaCl	Index.
1	*								*	2.7
2		*							*	3.0
3			*						*	0.2
4				*					*	2.2
5					*				*	1.56
6						*			*	1.4
7							*		*	0.9
8								*	**	0.0
9									**	0.08
10	**									3.5
11	*		*							2.56
12	*			*						3.1
13	*				*					1.53
14	*					*				2.2
15	*							*	*	1.6
16			*			*				1.13
17			*				*			0.12
18						*		*	*	0.93
19		*			*					1.6

Remarks. Human Leucocytes. ¼ hour incubated. Tuberculous serum from advanced case. Serum treated with bacteria at -1°, both serum & bacteria cooled to -1° for 2 minutes before mixing. Centrifuged in cold.

EXPERIMENT No LII. 27-4-'08.

No	Normal Human Serum diluted 1-50th.	N.H.S. 1-100th.	N.H.S. 1-500th.	N.H.S. 1-1000th.	N.H.S. 1-5000th.	N.H.S. 1-10,000th.	N.H.S. 1-20,000th.	N.H.S. 1-50,000th.	N.H.S. 1-100,000th.	NaCl.	Index. All cells counted.	Index. no cells counted over 10.	Index. Percentage of noughts.
1	*										5.6	2.0	35%
2		*									5.0	3.6	13
3			*								6.53	4.06	7
4				*							5.52	3.2	10
5					*						7.16	3.4	15
6						*					5.5	3.76	26
7							*				3.95	2.55	32
8								*			4.8	2.8	32
9									*		6.9	3.3	33
10										*	3.03	2.26	33
11					(*Incubated for ½ hour*						2.3	2.0	40
12					*at 37°. Kept 3 hours*						2.56	2.16	35
					at Room Temperature)								

Remarks. Human leucocytes. Incubated 3 hours.
Dilutions made with 0.85% NaCl.

EXPERIMENT No LIII. 29-4-'08.

No	Normal Human Serum.	N.H.S. diluted 1-50th in 1.2% NaCl.	N.H.S. 1-100th in 1.2% NaCl.	N.H.S. 1-500th in 1.2% NaCl	N.H.S. 1-1,000th in 1.2% NaCl.	N.H.S. 1-5,000th in 1.2% NaCl	N.H.S. 1-10,000th in 1.2% NaCl.	N.H.S. 1-20,000th in 1.2% NaCl.	N.H.S. 1-50,000th in 1.2% NaCl	N.H.S. 1-100,000th in 1.2% NaCl.	NaCl. 1.2%	Time of incubation	Index.	Percentage of noughts.
1	*											¼ hr	3.7	10%
2	*											1	5.53	3
3	*											4	(1.9)	35
4	*											5	2.7	16
5		*										¼	1.5	42
6		*										1	2.1	33
7		*										2	2.62	15
8		*										5	2.4	25
9			*									5	1.5	53
10				*								5	1.2	55
11					*							5	1.2	57
12						*						5	1.4	45
13							*					5	1.1	47
14								*				5	1.5	43
15									*			5	p.5	70
16										*		5	0.96	63
17											*	5	0.3	80

Remarks. Human leucocytes Human Serum fresh, all dilution in 1.2% NaCl, which according to Wright prevents spontaneous phagocytosis. 4 hours incubation of No 3 at Room Temperature.

N.B. There was no appearance of the Bacteria being broken up or digested inside the cells to account for the lower figures after 5 hours.

EXPERIMENT No LIV. 30-4-'08

No	Normal Human Serum I	Tub: Human Serum II	I. heated 56° for ½ hour.	II heated 56° for ½ hour	N.H.S. diluted 1-10,000 one day old.	N.H.S. diluted 1-50,000 one day old	N.H.S. diluted 1-100,000 one day old.	NaCl, 1.2%	Time of Incubation	Index	Percentage of noughts.
1	*							*	¼ hour	2.5	17%
2	*							*	1½ hours	5.7	3
3	*							*	3½ do	4.9	10
4		*						*	¼ do	2.42	22
5		*						*	1½ do	5.5	5
6		*						*	3½ do	3.63	10
7			*					*	¼ hour	0.2	83
8				*				*	¼ do	0.63	80
9	*		*						¼ do	2.08	23
10	*			*					¼ do	1.45	40
11		*	*						¼ do	1.8	35
12		*		*					¼ do	1.7	30
13					**				3½ hours	0.2	80
14						**			3½ do	0.4	80
15							**		3½ do	0.1	90
16								**	¼ hour	0.1	93
17								**	3½ hours	0.03	96

Remarks. Human leucocytes. Diluted Serum one day old, made with 1.2%NaCl. After 3½ hours incubation no noticeable breaking up of the injected bacteria.

Notice:- T.B. heated much above normal heated, also greater antagonism of T.B. heated, Notice too that, while 1, 4, 7 and 8 have 1.2% NaCl in them to reduce spontaneous phagocytosis, and 9, 10, 11, and 12 none, yet antagonism noticeable.

Very good slides, 30 to 90 leucocytes counted in each.

EXPERIMENT No LV. 4-5-'08.

No	I N.H.S.	II N.H.S.	III N.H.S.	IV N.H.S.	V N.H.S.	VI N.H.S.	VIA N.H.S.	VIIA N.H.S.	XI N.H.S.	I 56°	II 56°	III 56°	IV 56°	V 56°	VI 56°	VIA 56°	VIIA 56°	IX 56°	0.85% NaCl	Index.
1	*																		*	1.86
2		*																	*	1.36
3			*																*	1.43
4				*															*	1.4
5					*														*	1.23
6						*													*	1.8
7							*												*	2.03
8								*											*	1.72
9									*										*	1.53
10										*									*	0.16
11											*								*	0.1
12												*							*	0.13
13													*						*	0.1
14														*					*	0.03
15															*				*	0.03
16																*			*	0.1
17																	*		*	0.13
18																		*	*	0.36
19	*									*									*	1.5
20	*											*							*	1.7
21		*									*								*	1.46
22			*									*							*	2.0
23				*									*						*	0.97
24					*									*					*	1.0
25						*									*				*	1.4
26							*									*			*	2.26
27								*									*		*	0.73
28									*									*	*	1.13
29																			**	0.03

Remarks. Human leucocytes. ¼ hour incubated.
Serums heated ½ hour

EXPERIMENT No LVI. 9-5-'08

No	Normal Human Serum I	Tub: Human Serum II	Tub: H. S. III	Tub: H. S. IV	Tub: H. S. V	Tub: H. S. VI	I. -1° for ½ hour	II. -1° for ½ hour	III. -1° for ½ hour	IV. -1° for ½ hour	V. -1° for ½ hour	VI. -1° for ½ hour	I treated with T.B. for ½ hour at -1°	T.B. treated with I for ½ hour at -1°	1.2% NaCl	Index.
1	*														*	1.44
2		*													*	1.13
3			*												*	1.3
4				*											*	1.0
5					*										*	1.4
6						*									*	1.0
7							*								*	1.3
8								*							*	-
9									*						*	0.8
10										*					*	1.0
11											*				*	1.44
12												*			*	0.9
13													*		*	0.6
14														*	**	0.0
15															**	0.0
16	*						*									1.33
17	*												*			1.06
18	*													*	*	1.0
19	*										*					1.2
20					*					*						0.96
21						*					*					0.93
22							*					*				-

Remarks. Human leucocytes. ¼ hours incubated. NaCl used 1.2%. Serum and Bacteria put in ice and K_2SO_4 mixture one minute before mixing. Centrifuged in ice mixture.

EXPERIMENT No LVII. 13-5-08.

No	Normal Human Serum	N. H. S. II	Tub: Human Serum III	Tub: Human Serum IV	Tub: H. S. V.	I heated 56° for ½hour.	I heated 53.5° for ½ hour	I heated 51° for ½ hr.	V heated 56° for ½ hr	V heated 53.5° for ½ hour	V heated 51° for ½ hr	I diluted with 1.2% NaCl 1/20	NaCl 1.2%	Index
1	*												*	1.56
2		*											*	1.7
3			*										*	1.33
4				*									*	1.23
5					*								*	1.56
6						*							*	0.4
7							*						*	0.3
8								*					*	0.95
9									*				*	0.3
10										*			*	0.5
11											*		*	0.9
12												*	*	0.8
13	*					*								1.5
14	*						*							0.96
15	*							*						2.0
16					*				*					0.85
17					*					*				1.44
18					*						*			1.9
19						*						*		0.92
20									*			*		0.68
21													**	0.03

Remarks. Human Leucocytes. ¼ hour incubated. Most beautiful slides. In many 100 leucocytes counted.

EXPERIMENT No LVIII. 14-5-08.

No	Normal Human Serum	N.H.S. diluted 1-20th with 0.85% NaCl	N.H.S. heated 56° for ½ hour	N.H.S. heated 53° for ½ hour	Tub: Human Serum 56° for ½ hour	Tub: Human Serum 53° for ½ hour	Tub: Human Serum	NaCl 0.85%	Index
1	*							*	2.5
2		*						*	0.54
3			*					*	0.06
4				*				*	0.3
5					*			*	0.16
6						*		*	1.05
7							*	*	2.2
8								**	0.06
9		*	*						0.68
10		*		*					0.26
11		*			*				0.41
12		*				*			0.62
13	**								2.3
14		**							0.7

Remarks Human Leucocytes. ¼ hour incubated.

Tuberculous case early.

EXPERIMENT LIX. 15-5-08.

No	N. H. S.	N.H.S. ½ in 0.35% NaCl.	N.H.S. + $\frac{N}{2}$ HNO_3 1-1 neutralised after ¼ hour by NaOH	N.H.S. + $\frac{N}{3}$ HCl 1-1 neut: after ½ hour by NaOH.	N.H.S. + $\frac{N}{2}$ HNO_3 1-1 neut: after 1 hour by NaOH.	N.H.S. + $\frac{N}{3}$ HCl 1-1 neut: after 2 hours by NaOH.	N.H.S. + $\frac{N}{2}$ HNO_3 1-1 neut: after 3 hours by NaOH.	N.H.S. + $\frac{N}{2}$ HNO_3 1-1	N.H.S. + $\frac{N}{2}$ HNO_3 4-1	N.H.S. + $\frac{N}{10}$ HNO_3 4-1	N.H.S. + $\frac{N}{20}$ HNO_3 4-1	N.H.S. + $\frac{N}{40}$ HNO_3 4-1	N.H.S. + $\frac{N}{100}$ HNO_3 4-1	N.H.S. + $\frac{N}{3}$ HCl 1-1	N.H.S. + $\frac{N}{3}$ HCl 4-1	N.H.S. + $\frac{N}{3}$ HCl + $\frac{N}{3}$ NaOH 1-1-1 at once	N.H.S. + $\frac{N}{2}$ HNO_3 + $\frac{N}{2}$ NaOH 1-1-1 at once	0.35% NaCl	Index
1	*																	*	1.5
2		**																	1.62
3			**																0.15
4				**															0.12
5					**														0.15
6						**													0.2
7							**												0.1
8								**											0.06
9									**										0.16
10										**									1.6
11											**								1.5
12												**							1.8
13													**						1.65
14														**					0.05
15															**				1.4
16																**			0.15
17																	**		0.07
18																		**	0.1

Remarks. Human Leucocytes ¼ hour incubated.

neut = neutralised.

EXPERIMENT No LX. 16-5-'08.

No	Normal Human Serum I.	N.H.S. diluted 1/20 with 0.85 NaCl.	Tub: Human Serum II	Tub: Human Serum III	I heated 56° for ½ hour	I. heated 53° for ½ hour.	II. heated 56° for ½ hour	II. 53° for ½ hour	III heated 56° for ½ hour.	III. heated 53° for ½ hour	NaCl 0.85%	Index
1	*										*	2.13
2		*									*	0.6
3			*								*	2.0
4				*							*	1.8
5					*						*	0.3
6						*					*	0.33
7							*				*	0.3
8								*			*	0.53
9									*		*	0.1
10										*	*	0.23
11											**	0.1
12		*			*							0.8
13		*				*						0.5
14		*					*					0.75
15		*						*				1.0
16		*							*			0.43
17		*								*		0.6

Remarks. Human Leucocytes. ¼ hour incubated.

Tuberculous cases, moderately severe.

EXPERIMENT No LXI. 18-5-'08.

No	N. H. S. I	N.H.S. ½ in 0.85% NaCl	N. H. S. II	N.H.S. I + $\frac{N}{3}$ HCl 1-1	N.H.S. I + $\frac{N}{3}$ HCl 4-1	N.H.S. I + $\frac{N}{3}$ HCl 1-1 after ½ hour neutralised with NaOH	N.H.S. II + $\frac{N}{3}$ HCl 1-1	N.H.S. II + $\frac{N}{3}$ HCl 4-1	N.H.S. II + $\frac{N}{3}$ HCl 1-1 after ½ hour neutralisation with NaOH	NaCl 0.85%	Index.
1	*									*	2.36
2		**									2.2
3			*							*	2.13
4				**							0.06
5					**						2.0
6						**					0.5
7							**				-
8								**			1.5
9									**		0.4
10										**	0.05

Remarks. Human Leucocytes. ¼ hour incubated.

EXPERIMENT No LXII. 19-5-'08.

No	Normal Human Serum I	Tub: Human Serum II	Normal Rabbit Serum III	N. Rabbit Serum diluted 1-12th with 1.2% NaCl.	I. heated 60° for ½ hour	I. heated 56° for ½ hour	I. heated 53° for ½ hour	II. heated 60° for ½ hour	II. heated 56° for ½ hour	II. heated 53° for ½ hour	III. heated 60° for ½ hour	III. heated 56° for ½ hour	NaCl 1.2%	Index.	
1	*												*	1.5	
2		*											*	1.4	
3			*										*	1.3	
4				*									*	0.7	
5					*								*	0.1	
6						*							*	0.25	
7							*						*	0.2	
8								*					*	0.16	
9									*				*	0.3	
10										*			*	0.3	
11											*		*	0.06	
12												*	*	0.16	
13													**	0.06	
14				**										0.73	
15				*	*									0.56	
16				*		*								0.46	0.3
17				*			*							0.66	0.6
18				*				*						0.75	0.73
19				*					*					-	0.4
20				*						*				1.05	0.46
21				*							*			0.6	
22				*								*		0.5	

Duplicate Slides.

Remarks. Human Leucocytes. ¼ hour incubated.

Tuberculous case early.

EXPERIMENT No LXIII. 25-5-'08.

No	Normal Human Serum I	Tub: H. S. II	Tub: H. S. III	Normal Rabbit Serum IV	Normal Rabbit 1-12th in 1.2% NaCl.	I. heated 60° for ½ hr	I. heated 56° for ½ hour	II. heated 60° for ½ hr	II. heated 56° for ½ hr	III. heated 60° for ½ hr	III. heated 56° for ½ hr	IV. heated 60° for ½ hr	IV. heated 56° for ½ hr	1.2% NaCl	Index.	Percentage of noughts.
1	*													*	5.4	0
2		*												*	-	-
3			*											*	5.5	10
4				*										*	4.0	10
5					*									*	2.3	25
6					**										2.5	40
7						*								*	0.1	90
8							*							*	0.2	86
9								*						*	0.8	85
10									*					*	1.3	55
11										*				*	0.5	73
12											*			*	0.23	50
13												*		*	0.2	55
14													*	*	0.9	80
15					*	*									2.55	40
16					*		*								2.13	55
17					*			*							2.6	22
18					*				*						1.13	35
19					*					*					1.8	20
20					*						*				2.23	17
21					*							*			1.9	26
22					*								*		2).1	23
23														**	0.1	90

Remarks. Human Leucocytes. ¼ hour incubated
Tub: cases. III very bad. IV apparently recovered (disease arrested).

EXPERIMENT No LXIV. 26-5-'08.

No	Normal Human Serum I	Normal Rabbit Serum II	Tub: Human Serum III	Tub: Human Serum IV	I. heated for ½h. 56°	II. heated for ½h.56°	III.heated for ½h.56°	IV. heated for ½h.56°	NaCl, 1.2%	Index.	Percentage of Noughts.
1	*									4.09	3%
2		*								3.0	0
3			*							3.13	10
4				*						4.2	10
5					*					0.2	86
6						*				0.4	77
7							*			0.7	80
8								*		0.3	75
9									**	0.06	93
10	*				*					2.75	14
11	*					*				1.84	30
12	*						*			2.5	23
13	*							*		2.6	23
14	**									4.3	0

Remarks. Human Leucocytes. ¼ hour incubated.
Case III very bad. No IV disease arrested.

EXPERIMENT No LXV. 27-5-'08.

No	Normal Human Serum I	N. H. S. II	Tub: Human S. III	Tub: H. S. IV	Normal Guineapig Serum	N. G. S. 1-20th	N.G.S. heated 60° for ½ hour.	N.G.S. heated 56° for ½ hour.	I. heated 56° for ½ hour	III. heated 60° for ½ hr.	III. heated 56° for ½ hr.	NaCl., 1.2%	Index.	Percentage of Noughts.	
1	*											*	1.54	38%	
2		*										*	2.2	10	
3			*									*	2.06	23	
4				*								*	1.56	36	
5					*							*	1.6	25	
6						*						*	0.32	70	
7							*					*	0.26	83	
8								*				*	0.52	80	
9									*			*	0.23	86	
10										*		*	0.16	86	
11											*	*	0.23	83	
12	*				*								1.2	33	
13	*						*						1.06	53	
14	*								*				1.0	40	
15	*										*		1.26	43	Index
16					*		*						1.0	43	0.96 56% 2 specimens made
17					*			*					1.23	46	
18						**							0.53	70	
19						*	*						0.1	90	
20						*		*					0.38	75	
21						*			*				0.56	66	
22						*				*			0.63	73	
23						*					*		0.45	78	
24	**												2.15	33	
25											**		0.1	90	

Remarks. Human Leucocytes. ¼ hour incubated.
Serums heated ½ hour

EXPERIMENT No LXVI. 28-5-'08.

No	Normal Human Serum I	N. H. S. II	Tub: Human Serum III	Tub: Human Serum IV.	Normal Guineapig Serum	N.G.S. diluted 1-20th with 1.2% NaCl.	N.G.S. heated 60° for ½ hour	N. H. S. I heated 56° for ½ hour.	Tub: H.S. III, heated 56° for ½ hour	NaCl 1.2%	Index.	Percentage of Noughts.
1	*									*	1.2	35%
2		*								*	1.2	36
3			*							*	1.1	53
4				*						*	1.3	33
5					*					*	1.62	30
6						*				*	0.26	83
7							*			*	0.2	83
8								*		*	0.06	93
9									*	*	0.1	90
10										**	0.03	96
11	*						*				0.54	70
12	*							*			0.5	75
13	*								*		0.83	43
14					*		*				0.4	70
15						*	*				0.13	93
16						*		*			0.0	100
17						*			*		0.06	93
18						**					0.2	80

Remarks. Human Leucocytes. ¼ hour incubated.
Serums heated ½ hour

EXPERIMENT No LXVII. 29-5-'08.

No	Normal Human Serum I	N. H. S. II	Tub: Human Serum III	Tub: H. S. IV.	I diluted 1-20th with 1.2% NaCl	I. heated for ½ hour 56°	II. heated for ½ hour 56°	III. heated for ½ hr 56°	IV. heated for ½ hr 56°	NaCl 1.2%	Index.	Percentage of Noughts.
1	*									*	1.27	28%
2		*								*	0.9	42
3			*							*	1.14	36
4				*						*	1.06	46
5					*					*	0.2	88
6						*				*	0.06	96
7							*			*	0.1	93
8								*		*	0.36	83
9									*	*	0.33	83
10					*	*					0.14	90
11					*		*				0.2	87
12					*			*			0.22	90
13					*				*		0.4	70
14	*					*					0.66	58
15	*						*				1.02	47
16	*							*			0.38	70
17	*								*		0.86	53
18					**						0.43	80
19									**		0.03	96

Remarks. Human Leucocytes. ¼ hour incubated.

EXPERIMENT No LXVIII. 30-5-'08

No	Normal Human Serum I.	N. H. S. II.	Tub: Human Serum III	Tub: H. S. IV	I. diluted 1-20th with 1.2% NaCl	I. heated 65° for ½ hour	II heated 65° for ½ hour	III. heated 65° for ½ hour	IV. heated 65° for ½ hour	NaCl, 1.2%	Index	Percentage of Noughts.
1	*									*	0.75	55%
2		*								*	1.0	48
3			*							*	0.6	58
4				*						*	0.72	68
5					*					*	0.12	85
6						*				*	0.03	96
7							*			*	0.02	96
8								*		*	0.06	93
9									*	*	0.2	82
10	*					*					0.45	75
11	*						*				0.1	92
12	*							*			0.31	77
13					*	*					0.1	90
14					*		*				0.05	95
15					*			*			0.06	93
16					*				*		0.26	90
17					**						0.1	90

Remarks. Human Leucocytes. ¼ hour incubated.
Serum III and IV from bad cases.

EXPERIMENT No LXIX. 1-6-'08.

No	Normal Human Serum I	N. H. S. II	Tub: H. S. III	Tub: H. S. IV	I. diluted 1-20th with 1.2% of NaCl	I. heated 56° for ½hour	II. heated 56° for ½ hour	III. heated 56° for ½ hour	IV. heated 56° for ½ hour	I. heated 65° for ½ hour	NaCl, 1.2%	Index.	Percentage of Noughts.
1	*										*	0.82	56%
2		*									*	0.73	73
3			*								*	0.53	60
4				*							*	1.06	43
5					*						*	0.08	92
6						*					*	0.2	86
7							*				*	0.06	96
8								*			*	0.3	72
9									*		*	0.1	90
10										*	*	0.03	96
11	*					*						0.7	64
12	*									*		0.43	70
13					*	*						0.3	80
14					*		*					0.05	97
15					*			*				0.25	90
16					*				*			0.25	90

Remarks. Human Leucocytes. ¼ hour incubated.

No III, very bad case of Tuberculosis.

EXPERIMENT No LXX. 2-6-'08.

No	Normal Human Serum I	N. H. S. II	Tub: H. S. III	Tub: H. S. IV	I. diluted 1-20th with 1.2% NaCl.	I. heated 56° for ½ hour.	II. heated 56° for ½ hour.	III. heated 56° for ½ hour.	IV. heated 56° for ½ hour.	NaCl, 1.2%	Index.	Percentage of Noughts.
1	*									*	0.7	55%
2		*								*	0.73	56
3			*							*	0.96	63
4				*						*	0.96	53
5					*					*	0.2	85
6						*				*	0.03	96
7							*			*	0.03	96
8								*		*	0.2	97
9									*	*	0.17	94
10					**						0.06	96
11					*	*					0.0	100
12					*		*				0.03	96
13					*			*			0.25	85
14					*				*		0.26	70
15	*					*					0.72	70
16	*						*				0.66	66
17	*							*			0.55	72

Remarks. Human Leucocytes. ¼ hour incubated.
III and IV bad cases.

EXPERIMENT No LXXI. 3-6-'08.

No	Normal Human Serum I	I dilutted 1-20th with 1.2% NaCl.	I dilutted ½ heated 75° for ½ hour.	I. ½ , heated 70° for ½ hour.	I. ½, heated 65° for ½ hour.	I. ½, heated 60° for ½ hour.	I. ½, heated 56° for ½ hour.	½dil: N.H.S. II heated 70° for ½ hour	N.H.S.II ½, heated 65° for ½ hour.	II. ½, heated 60° for ½ hour.	Tub: H.S. dil: ½ , heated 60° for ½ hour.	Tub: H.S. dil: ½, heated 56° for ½ hour	NaCl, 1.2%	Index.
1	*												*	1.55
2		*											*	0.36
3					*								*	0.03
4							*						*	0.1
5								*					*	0.03
6											*		*	0.3
7												*	*	0.3
8	*		*											1.54
9	*			*										-
10	*				*									0.7
11	*							*						1.37
12	*								*					1.12
13	*										*			1.6
14		*	*											0.4
15		*		*										0.13
16		*			*									0.13
17		*				*								0.33
18		*					*							0.23
19		*						*						0.13
20		*							*					0.0
21		*								*				0.3
22		*									*			0.33
23		*										*		0.3
24													**	0.03

Remarks. Human Leucocytes. ½ hour incubated. Tub: case slight, arrested. All dilutions with 1.2% NaCl. I. 75° and 70° and II, 70° partially coagulated.

EXPERIMENT No LXXII. 4-6-'08.

No.	Normal Human Serum I	N. H. S. diluted 1-20th	N.H.S.I.heated 65° for ½ hour.	N.H.S.I.heated 60° for ½ hour.	N.H.S.I.heated 56° for ½ hour.	N.H.S.II. heated 70° for ½ hour.	N.H.S.II.heated 65° for ½ hour.	N.H.S.II.heated 56° for ½ hour.	Tub: H.S.heated 60° for ½ hour	Tub: H.S.heated 56° for ½ hour.	NaCl, 1.2%	Index.
1	*										*	1.0
2		*									*	0.3
3			*								*	0.03
4				*							*	0.03
5					*						*	0.1
6						*					*	0.03
7							*				*	0.0
8								*			*	0.12
9									*		*	0.06
10										*	*	0.14
11		*	*									0.0
12		*		*								0.0
13		*			*							0.03
14		*				*						0.03
15		*					*					0.1
16		*						*				0.16
17		*							*			0.05
18		*								*		0.27

Remarks. Human Leucocytes. ¼ hour incubated.

N.H.S. diluted with 1.2% NaCl. Tuberculosis slight.

EXPERIMENT No LXXIII. 5-6-'08.

No	Normal Human Serum I	N.H.S. diluted 1-20th with 1.2% NaCl.	N.H.S.I. heated 65° for ½ hour.	N.H.S.I. heated 56° for ½ hour.	N.H.S.II. heated 65° for ½ hour.	N.H.S.II. heated 56° for ½ hour	Tub: H.S.III. heated 65° for ½ hour	Tub: H.S.IV. heated 65° for ½ hour	NaCl, 1.2%	Index.
1	*									1.23
2		*								0.22
3			*							0.03
4				*						0.0
5					*					0.0
6						*				0.18
7							*			0.2
8								*		0.2
9		*	*							0.0
10		*		*						0.03
11		*			*					0.1
12		*				*				0.06
13		*					*			0.06
14		*						*		0.0
15	*		*							1.26
16	*				*					0.84
17	*						*			0.3
18	*							*		0.8
19	*			*						0.7

Remarks. Human Leucocytes. ¼ hour incubated.

Tub: III bad, IV. slight.

EXPERIMENT No LXXIV. 6-6-'08

No	Normal Human Serum I	I.diluted 1-20th with 1.2% NaCl.	I.heated 65° for ½ hr.	I.heated 56° for ½ hr	II.N.H.S.heated 65° for ½ hour.	II.heated 56° for ½ hr	Tub:H.S.III. 65° for ½ hour.	Tub:H.S.IV. 65° for ½ hour.	NaCl, 1.2%	Index.
1	*								*	1.5
2		*							*	0.4
3			*						*	0.03
4				*					*	0.25
5					*				*	0.0
6						*			*	0.25
7							*		*	0.06
8								*	*	0.06
9	*		*							0.76
10	*			*						1.2
11	*				*					0.86
12	*						*			0.5
13	*							*		0.33
14		*		*						0.4
15		*			*					0.3
16		*				*				0.65

Remarks. Human Leucocytes. ¼ hour incubated.

EXPERIMENT No LXXV. 29-6-'08.

No	Normal Human Serum.	N.H.S. 56° for ½ hour	Tub:H.S.56° for ½ hr.	N.H.S. 60° for ½ hr.	NaCl, 1.2%	Index.
1	*				*	1.3
2		*			*	0.08
3			*		*	0.1
4				*	*	0.1
5					**	0.0
6	*	*				0.93
7	*		*			0.83
8	*			*		0.26

Remarks. Human leucocytes. ¼ hour incubated T.B. Bacilli as usual.

EXPERIMENT No LXXVI. 28-8-'08.

No	Normal Human Serum	N.H.S. dialysed for 3 hours.	N.H.S. treated with Bacteria at 0°.	N.H.S. treated with Bacteria at 0°. then heated 56°.	NaCl. 0.85%	Index
1	*				*	3.72
2		*			*	0.7
3			*		*	1.8
4				*	*	0.2
5					**	0.2
6	*			*		3.83

Remarks. Human Leucocytes. ½ hour incubated.

Collodion sac used for dialysing.

Bacteria and Serum cooled before mixing at 0°.

After treatment centrifuged in the cold.

EXPERIMENT No LXXVII. 29-8-'08.

No	Normal Human Serum	N.H.S. heated 56°	N.H.S. treated with Bacteria at 0°	N.H.S. Control, 5 days old.	N.H.S. dialysed 5 days.	Tubercle Bacteria treated with N.H.S. at 0°.	NaCl exterior to dialysing sac after 5 days.	NaCl, 0.85%	Index.
1	*							*	2.7
2		*						*	0.15
3			*					*	0.3
4				*				*	2.13
5					*			*	0.2
6						*		*	0.05
7							*	**	0.03
8								**	0.06
9	*	*							2.06
10	*		*						1.98
11	*				*				2.5
12	*					*		*	2.45
13	*						*		2.2
14		*	*						0.2
15		*			*				0.25
16					*		*		0.13
17			*			*		*	0.26

Remarks. Human Leucocytes. ¼ hour incubated.
Collodion sac used for dialysis.

EXPERIMENT No LXXVIII. 31-8-'08.

	N. H. S. diluted with 0.85% NaCl.										
No	1/50	1/100	1/500	1/1000	1/5000	1/10000	1/20000	1/50000	1/100,000	0.85% NaCl	Index.
1	*										15.9
2		*									14.3
3			*								13.3
4				*							14.1
5					*						11.13
6						*					12.38
7							*				12.33
8								*			8.5
9									*		8.9
10										*	6.0

Remarks. Human Leucocytes. 3 hours incubation.

EXPERIMENT No LXXIX. 5-10-'08.

No	N. H. S. diluted with 0.85% NaCl.								0.85% NaCl	Index.
	1/50	1/100	1/500	1/1000	1/5000	1/10000	1/20000	1/50000	1/100,000	
1	*									4.9
2		*								4.55
3			*							5.6
4				*						3.83
5					*					3.63
6						*				3.4
7							*			2.9
8								*		3.0
9									*	1.8
10										1.43

Remarks. Human Leucocytes. 3 hours incubation.

EXPERIMENT No LXXX. 27-6-'08

No	N.H.S. 15 hours old.	N.H.S. ¼ in 1.2% NaCl	N.H.S. heated 56°	Tub: H.S. heated 56°	N.H.S. heated 56°	Tub: H.S. heated 56°	N.H.S. dialysed 15 hours.	NaCl, 1.2%	Index.	
1	*							*	14.5	
2		*						*	3.3	
3			*					*	6.46	
4				*				*	3.06	
5					*			*	3.86	
6						*		*	2.06	
7							*	*	0.86	
8								**	0.8	
9	*			*					10.7	
10	*				*				6.6	
11	*						*		0.9	
12		*	*						4.6	
13		*		*					1.83	
14			*				*		1.55	
15	*							*	25.0	
16	*							*	38.6	
17	*							*	-	

Remarks Human Leucocytes. Incubation ¼ hour for I-14
¾ do 15
1 do 16
1½ do 17

Serums heated for ½ hour

Pyocyaneus Bacteria.

EXPERIMENT No LXXXI. 1-7-'08.

No	N. H. S. I	N.H.S.I. diluted ¼ with 1·2% NaCl	N.H.S. I. heated 56°	N.H.S.II. heated 56°	N.H.S.III.heated 56°	N.H.S.IV. heated 56°	N.H.S. V. heated 56°	N.H.S.VI. heated 56°	N.H.S.VII.heated 56°	N.H.S.III.heated 60°	N.H.S. V. heated 60°	N.H.S.VI. heated 60°	N.H.S.VII.heated 60°	NaCl, 1.2%	Index.
1	*													*	3.16
2		*												*	1.0
3			*											*	0.4
4				*										*	0.2
5					*									*	0.93
6							*							*	0.9
7										*				*	0.2
8											*			*	0.2
9		*	*												1.36
10		*		*											0.93
11		*			*										0.55
12		*				*									0.83
13		*					*								0.9
14		*						*							0.1
15		*							*						0.2
16		*								*					0.6
17		*									*				0.35
18		*										*			0.6
19		*											*		0.65
20														**	0.03
21															

Remarks. Human Leucocytes. Incubated ¼ hour

Pyocyaneus Bacteria

Serums heated ½ hour

EXPERIMENT No LXXXII. 30-7-'08.

No	Normal Human Serum.	N. H. S. $\frac{1}{4}$	N.H.S. heated 56°	N.H.S. treated with Bacteria at 37°	Pyocyaneus Culture Filtrate	Pyocyaneus Bacteria treated with N.H.S. at 37°	Pyocyaneus Bacteria treated with heated 56° Serum at 37°	NaCl, 0.85%	Index.
1	*							*	3.6
2		*						*	0.6
3			*					*	0.6
4				*				*	0.53
5					*			*	0.06
6						*		**	0.5
7							*	**	0.2
8								**	0.1
9		*	*						0.4
10		*			*				0.0
11		*				*		*	0.25
12		*					*	*	0.5
13			*	*					1.2
14	*				*				0.4
	NHS	NHS$\frac{1}{4}$	$\frac{1}{20}$	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{5000}$		
15	*								8.6
16		*							2.1
17			*						1.7
18				*					0.83
19					*				1.26
20						*			0.76
21							*		0.63
22								*	0.3

Remarks. Human Leucocytes. $\frac{1}{4}$ hour incubated.
Pyocyaneus Bacteria.
Heating & other treatment of Serum for $\frac{1}{2}$ hour

EXPERIMENT No LXXXIII. 31-7-'08.

No	Normal Human Serum	N.H.S. treated with Bacteria at 37°	N.H.S. heated 56°	Pyocyaneus Culture Filtrate.	Pyocyaneus Bacteria treated with N.H.S. at 37°	Pyocyaneus Bacteria treated with heated 56° Serum at 37°	NaCl, 1.2%	Index
1	*						*	0.83
2			*				*	0.2
3				*			*	0.03
4					*		**	0.23
5						*	**	0.1
6							**	0.03
7	*		*					0.8
8	*			*				0.3
9	*				*		*	0.64
10	*					*	*	0.85
11		*					*	0.3

D I L U T E D .

		1/4	1/20	1/100	1/500	1/1000	1/5000	
1	*							9.32
2		*						1.3
3			*					0.6
4				*				-
5					*			1.0
6						*		0.7
7							*	0.3
8								0.3

Remarks. Human Leucocytes. ¼ hour incubated Pyocyaneus Bacteria

EXPERIMENT No LXXXIV. 1-8-'08.

No	Normal Human Serum I	N.H.S. ¼ in 1.2% NaCl II.	N.H.S. heated 56°	N.H.S. I. 60°	N.H.S. II 60°	N.H.S. III 60°	N.H.S. IV. 60°	Pyocyaneus Culture Filtrate	NaCl, 1.2%	Index.
1	*								*	1.6
2		*							*	1.0
3			*						*	0.43
4				*					*	0.6
5					*				*	0.4
6						*			*	0.6
7							*		*	0.43
8								*	*	0.03
9									**	0.02
10		*	*							0.45
11		*		*						1.4
12		*			*					0.5
13		*				*				0.55
14		*					*			0.2
15	*			*						0.9
16	*				*					0.8
17	*					*				0.45
18	*						*			0.3
19	*							*		0.35
20		**								0.7

Remarks. Human Leucocytes. ¼ hour incubated.
Pyocyaneus bacteria. Serums heated ¼ hour

EXPERIMENT No LXXXV. 4-8-'08.

No	Normal Human Serum	N.H.S.¼ in 1.2% NaCl	N.H.S. heated 56°	N.H.S.treated with Bacteria at 0°	Pyocyaneus Bacteria treated with N.H.S. at 0°	NaCl, 1.2%	Index.
1	*					*	1.8
2		*				*	0.35
3			*			*	0.4
4				*		*	0.4
5					*	**	0.3
6						**	0.1
7	*		*				2.1
8	*			*			1.5
9	*				*	*	1.3
10		*	*				0.4
11		*			*	*	0.4
12			*	*			0.5
13				*	*		0.5

Remarks. Human Leucocytes. incubated ¼ hour Pyocyaneus Bacteria. Serum and Bacteria cooled before mixing at 0° and after treatment of ½ hour centrifuged in ice-mixture.

EXPERIMENT No LXXXVI. 5-8-'08.

No	Normal Human Serum.	N.H.S.¼ in 1.2% NaCl	N.H.S. heated 56°	N.H.S. treated with Bacteria at 0°	Pyocyaneus Bacteria treated with N.H.S. at 0°	Bacteria treated with N.H.S. at 37°	Bacteria treated with heated 56° serum at 37°	NaCl, 1.2%	Index.
1	*							*	11.3
2		*						*	0.5
3			*					*	0.8
4				*				*	0.56
5									-
6					*			*	0.26
7						*		*	1.26
8							*	*	0.7
9								**	0.4
10	*				*			*	9.6
11	*					*		*	-
12	*						*	*	6.2
13	**								16.0
14		*	*						0.06
15		*			*			*	0.53
16		*				*		*	1.66
17		*					*	*	0.8
18		**							1.4
19				*	*				0.6
20				*		*			-
21				*			*		6.2
22			*	*					0.36

Remarks. Human Leucocytes. ¼ hour incubated. Pyocyaneus Bacteria. Serum and Bacteria cooled before mixing at 0° and after ½ hour contact at 0° centrifuged in ice and separated.

EXPERIMENT No LXXXVII. 6-8-'08.

No	Normal Human Serum	N.H.S. ¼ in 1.2% NaCl	N.H.S. heated 56°	N.H.S. treated with Bacteria at 0°	Bacteria treated with N.H.S. at 37°	Bacteria treated with N.H.S. at 0°	Bacteria treated with heated 56° Serum at 37°	NaCl, 1.2%	Index.
1	*							*	1.1
2		*						*	0.5
3			*					*	0.6
4				*				*	0.6
5					*			**	0.35
6						*		**	1.0
7							*	**	0.3
8								**	0.3
9	*			*					1.23
10	*				*			*	1.1
11	*					*		*	1.3
12	*						*	*	1.2
13		*		*					0.4
14		*			*			*	0.45
15		*				*		*	1.1
16		*					*	*	0.4

Remarks. Human Leucocytes. ¼ hour incubated. Pyocyaneus bacteria. Bacteria and Serum cooled before mixing at 0°, after ½ hour contact centrifuged in ice mixture and separated.
Serums heated for ½ hour.

EXPERIMENT No LXXXVIII. 7-8-'08.

No	Normal Human Serum I	N.H.S.I. ¼ in 1.2% NaCl.	N.H.S.I. 56°	N.H.S.II. 56°	N.H.S.III.56°	N.H.S. IV.56°	N.H.S.II. 60°	N.H.S.III.60°	N.H.S.IV. 60°	N.H.S. V. 60°	N.H.S.VI. 60°	NaCl, 1.2%	Index.
1	*											*	12.13
2		*										*	1.0
3			*									*	2.15
4				*								*	1.73
5					*							*	1.73
6						*						*	2.8
7							*					*	1.43
8								*				*	1.3
9									*			*	2.4
10										*		*	3.6
11											*	*	-
12												**	0.3
13		*	*										1.1
14		*		*									0.8
15		*			*								0.5
16		*				*							1.76
17		*					*						2.0
18		*						*					1.6
19		*							*				-
20		*								*			1.43
21		*									*		0.4

Remarks Human Leucocytes. ¼ hour incubation.

Pyocyaneus bacteria.

Serums heated as always ½ hour

EXPERIMENT No LXXXIX. 9-8-'08.

No	Normal Human Serum I	N.H.S.I ¼ in 1.2% NaCl.	N.H.S. I. 56°	N.H.S. II. 56°	N.H.S. III. 56°	N.H.S. IV. 56°	N.H.S. I. 60°	N.H.S. II. 60°	N.H.S. III. 60°	N.H.S. IV. 60°	NaCl, 1.2%	Index.
1	*										*	5.2
2		*									*	1.4
3			*								*	1.7
4				*							*	2.1
5					*						*	1.63
6						*					*	1.2
7							*				*	0.8
8								*			*	1.2
9									*		*	1.43
10										*	*	1.45
11											**	0.5
12		*	*									3.6
13		*		*								3.74
14		*			*							1.64
15		*				*						2.66
16		*					*					0.2
17		*						*				1.55
18		*							*			2.4
19		*								*		1.4

Remarks. Human Leucocytes ½ hour incubated.

Pyocyaneus bacteria. Serums heated ½ hour

EXPERIMENT No XC. 27-8-'08.

No	Normal Human Serum I	N.H.S.I. ¼ in 1.2% NaCl.	N.H.S. I. 56°	N.H.S. II. 56°	N.H.S. III. 56°	N.H.S. IV. 56°	N.H.S. V. 56°	N.H.S. VI. 56°	N.H.S.I. 60°	N.H.S. II. 60°	N.H.S. III. 60°	N.H.S. V. 60°	N.H.S. VI. 60°	NaCl, 1.2%	Index.
1	*													*	10.0
2		*												*	5.08
3			*											*	2.85
4				*										*	2.3
5					*									*	2.33
6						*								*	2.0
7							*							*	2.8
8								*						*	3.4
9									*					*	2.8
10										*				*	2.08
11											*			*	2.5
12												*		*	2.05
13													*	*	2.0
14														**	1.8
15		*	*												2.5
16		*		*											3.6
17		*			*										1.9
18		*				*									1.2
19		*					*								3.2
20		*						*							2.0
21		*							*						3.0
22		*								*					3.0
23		*									*				-
24		*										*			2.0
25		*											*		-

Remarks. Human Leucocytes. ¼ hour incubated.
Pyocyaneus Bacteria. Serums heated ½ hour.

EXPERIMENT No XCI. 28-8-'08.

No	Normal Human Serum 1-10th	N.H.S. treated with Bacteria at 0°	N.H.S. treated with Bacteria at 0° then heated 56°	N.H.S. treated with Bacteria at 37°	N.H.S. treated with Bacteria at 37° then heated 56°	Bacteria treated with N.H.S. at 0°	Bacteria treated with N.H.S. at 37°	Bacteria treated with N.H.S. heated 56° at 37°	NaCl, 0.85%	Index.
1	*								*	5.16
2		*							*	7.15
3			*						*	2.12
4				*					*	1.55
5					*				*	1.55
6						*			**	1.05
7							*		**	-
8								*	**	3.93
9									**	0.83
10	*		*							4.8
11	*				*					2.2
12	*					*				3.6
13	*						*			3.5
14	*							*		2.26
	Diluted N.H.S. in 0.85% NaCl.									
		1/10	1/50	1/100	1/500	1/1000	1/2000	1/5000	1/10000	
1	*									12.4
2		*								9.0
3			*							8.9
4				*						6.8
5					*					9.2
6						*				5.3
7							*			8.6
8								*		6.8
9									*	5.5
10									*	1.45

Remarks. Human Leucocytes. ¼ hour incubated. Pyocyaneus bacteria. Ice experiment, same precautions taken as before.

EXPERIMENT No XCII 29-8-'08

No	Normal Human Serum	N. H. S. dialysed for 3 hours.	N.H.S. treated with Bacteria at 0°	N.H.S. heated 56°	NaCl, 0·85 %	Index
1	*					3.8
2		*				0.0
3			*			3.5
4				*		0.3
5					*	0.0
6	*	*				3.5
7	*		*			3.4
8	*			*		2.0
9		*		*		0.3
10			*	*		1.2

Remarks. Human Leucocytes. ½ hour incubated. Pyocyaneus Bacteria. Mixture of Serum and Bacteria treated at 0° for ½ hour and centrifuged in ice mixture.

Collodion sac used for dialysis.

EXPERIMENT No XCIII. 31-8-08.

No	Normal Human Serum	N.H.S. dialysed for 1 hour.	N.H.S. dialysed for 2 hours	N.H.S. dialysed for 3 hours.	NaCl, 0.85%	Index.	
1	*					3.33	
2		*				0.8	
3			*			1.33	
4				*		0.2	
5					*	0.1	

Remarks. Human Leucocytes. incubated ½ hour

Pyocyaneus Bacteria.

Collodion sac used.

EXPERIMENT No XCIV. 1-9-'08.

No	Normal Human Serum	N. H. S. dialysed 1 hour.	N. H. S. dialysed 2 hours.	N. H. S. dialysed 3 hours.	NaCl. 0.85%	Index.	
1	*				*	1.2	
2		*			*	1.16	
3			*		*	0.5	
4				*	*	0.1	
5					**	0.1	

Remarks. Human Leucocytes. ¼ hour incubated.
Paratyphoid bacteria. Collodion sac

No	Normal Human Serum I	Normal Human Serum II	Dialysed N.H.S. Int: of Reed Bag.	NaCl Ext: of same.	NaCl	EXPERIMENTS. XCV. 20-2-08 Index	XCVI 21-2-08 Index	XCVII 22-2-08 Index
1	*				*	1.6	1.33	0.8
2		*			*	1.53	1.06	0.9
3			*		*	1.3	0.6	0.36
4				*	*	0.03		
5	*			*		1.0	0.66	0.3
6					**	0.03		

Remarks. Human Leucocytes. Incubated ¼ hour.

20-2-08 Dialysis 2½ hours duration, Serum I
21-2-08 do 24 do do
22-2-08 do 2 days duration do.

No	Normal Human Serum I	Normal Human Serum II	Dialysed N.H.S. Int: of Reed Bag.	NaCl Ext: of Reed Bag.	NaCl.	EXPERIMENTS XCVIII 23-2-08 Index	XCIX 25-2-08 Index
1	*				*	1.0	2.3
2		*			*	1.33	1.5
3			*		*	1.23	
4	*			*			1.13

Remarks Human Leucocytes. Incubated ¼ hour

23-2-08 Dialysis 3 days duration, Serum I

25-2-08 do 5 do do

EXPERIMENT No C. 19-2-08

No	Normal Human Serum I	Normal Human Serum II	Dialysed N.H.S. II. Int: of Reed sac, 2½ days.	NaCl Ext: of Reed sac 2½ days.	NaCl.	Index.	Remarks.
1	*				*	1.6	Human Leucocytes. incubated ¼ hour. Serum II, 2½ days in Reed sac which was in equal quantity with Normal Saline in glass tube.
2		*			*	1.54	
3			*		*	1.3	
4				*	*	0.03	
5		*		*		0.96	
6					* *	0.06	

EXPERIMENT No CI. 26-1-'09.

No	N. H. S.	N.H.S.dialysed 1 hour.	N.H.S.dialysed 2 hours.	N.H.S.dialysed 3 hours	N.H.S.heated 56°	NaCl, 0.85%	Index.	Remarks.
1	*						5.2	Human Leucocytes. ¼ hour incubated. Paratyphoid (Schottmüller) bacteria. Collodion sac used.
2		*					3.5	
3			*				2.1	
4				*			1.0	
5					*		0.6	
6						*	3.3	
7				*	*		1.2	

EXPERIMENT No CII. 29-1-'09

No	N. H. S.	N. H. S. dialysed 1 hour.	N. H. S. dialysed 2 hours.	N. H. S. dialysed 3 hours	Index
1	*				1.05
2		*			0.5
3			*		0.7
4				*	0.3
5	0.85% NaCl alone				0.13

Remarks. Paratyphoid. Collodion sac used.
Human Leucocytes. incubated ¼ hour.

EXPERIMENT No CIII. 30-1-'09.

No	N. H. S.	N. H. S. dialysed 1 hour.	N. H. S. dialysed 2 hours	N. H. S. dialysed 3 hours.	NaCl.0.85%	Index.
1	*					2.43
2		*				2.13
3			*			0.8
4				*		0.4
5					*	-

Remarks. Paratyphoid. Collodion sac used for dialysing.
Human Leucocytes. ¼ hour incubated.

EXPERIMENT No CIV. 10-2-'09.

No	N. H. S.	N. H. S. dialysed 1 hour	N. H. S. dialysed 2 hours	N. H. S. dialysed 3 hours	NaCl. 0.85%	Index.	
1	*					3.1	
2		*				2.93	
3			*			0.75	
4				*		0.2	
5					*	0.2	

Remarks. Collodion sac. Human Leucocytes ¼ hour incubated Schottmüller's Paratyphoid.

EXPERIMENT No CV. 23-2-'09.

No	N. H. S.	N. H. S. heated 56°	N. H. S. treated with Bacteria at 0°	Bacteria treated with N.H.S. at 0°	NaCl. 0.85%	Index.	
1	*				*	9.05	
2		*			*	2.6	
3			*		*	5.0	
4				*	**	2.0	
5					**	0.62	
6		*	*			2.5	
7			*	*		2.1	

Remarks. Human Leucocytes. ¼ hour incubated.
Paratyphoid Bacteria (Schottmüller)

EXPERIMENT No CVI. 17-3-'09.

No	N. H. S. fresh.	N.H.S. 30 hours old, kept at 10-15°.	N.H.S. 27 days old, kept at 10-15°	N.H.S. heated 56° kept at 10-15°	N.H.S. treated with Bacteria at 0°	Mixture of N.H.S. and N.H.S. heated 56° kept for 30 hours at 37°	Mixture of N.H.S. and N.H.S. heated 56° kept for 30 hours at 10-15°	Mixture of N.H.S. and N.H.S. 56° each 30 hrs old mixed for ¼ hour at 10-15°	Tubercle Bacteria treated with N.H.S. at 0°	NaCl. 0.85%	Index.
1	*									*	3.4
2		*								*	3.23
3			*							*	1.1
4				*						*	0.52
5					*					*	0.98
6						**					2.3
7							**				1.93
8								**			0.9
9									*	**	0.2
10										**	0.1
11	*				*						1.4
12		*		*							1.07
13			*		*						1.55
14				*	*						0.4
15					*				*		0.88

Remarks. Human Leucocytes. ¼ hour incubated.
Tubercle Bacteria.

EXPERIMENT No CVII. 17-3-'09.

No	N. H. S. fresh.	N.H.S.30 hours old kept at 10-15°	N.H.S. 27 days old kept at 10-15°	N.H.S. heated 56°, 30 hours old	N.H.S. treated with Bacteria at 0°	Mixture of N.H.S. with N.H.S. heated 56° kept for 30 hours 37°	Mixture of N.H.S. with N.H.S. heated 56° kept for 30 hrs, 10-15°	Paratyphoid Bacteria treated with N.H.S. at 0°	NaCl. 0.85%	Index.
1	*								*	8.4
2		*							*	8.0
3			*						*	7.13
4				*					*	6.0
5					*				*	4.25
6						**				6.83
7							**			6.5
8								*	**	1.3
9									**	1.2
10	*				*					5.0
11		*		*						6.33
12			*		*					4.0
13				*	*					5.1
14					*			*		2.7

Remarks. Human Leucocytes. ¼ hour incubated.
Paratyphoid Bacteria.

EXPERIMENT No CVIII. 12-2-'09.

No	Normal Human Serum	N. H. S. treated with Bacteria at 0°	N.H.S. 25 days old	N.H.S. heated 56° for ½ hour.	Paratyphoid Bacteria treated with N.H.S. at 0°	NaCl. 0.85%	Index
1	*					*	7.32
2		*				*	5.5
3			*			*	8.5
4				*		*	3.5
5					*	*	0.6
6						**	0.45
7		*	*				8.0
8		*		*			4.62
9		*			*		6.0

Remarks. Paratyphoid Bacteria.
Human Leucocytes. ¼ hour incubated.
Bacteria and Serum cooled before mixing at 0°,
centrifuged in ice mixture after treatment.

My teachers in those subjects upon which this Thesis bears have been:--

In Tuberculosis

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In Pure Chemistry

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In Physical Chemistry

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In Physiological Chemistry

Professor Noel Paton, Glasgow.

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In Bacteriological Chemistry.

Dr. Friedmann, Berlin.

In Bacteriology

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Professor Levy, Strassburg.

In Immunity

Dr. Fornet, Strassburg, who has taught me the Method of Wright and with whom I have made a few of the foregoing experiments, namely, 1-6, 8, 9, 38, 40-43, and whose permission I now have to use them.